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# Longitudinal Predictors of Poor Outcome in Crohn's Disease

James IRWIN

BSc. MBChB

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QIMR Berghofer Medical Research Institute

# Abstract

Crohn's disease is a chronic inflammatory condition of the human gastrointestinal tract. It affects approximately one in five hundred individuals in Australia, predominantly young adults. It can cause significant morbidity from bowel obstruction, bowel perforation, perianal abscess or perianal fistula formation, or chronic bowel inflammation. A significant proportion of patients with Crohn's disease develop irreversible bowel damage, damage which requires surgical resection of the affected bowel to relieve symptoms. Medical therapies used to treat Crohn's disease modify the host immune system and reduce bowel inflammation, reducing symptoms of pain and diarrhoea, and possibly reducing progression to irreversible bowel damage.

Medical therapies increase the risk of opportunistic infection, the risk of developing skin cancer or lymphoma, and carry a risk of drug induced effects such as hepatitis or bone marrow suppression. These risks are likely to increase with increasing strength of immunosuppression.

Therapy in Crohn's disease needs to appropriately weigh risks and benefits for individual patients. Accurate and objective prediction of likely outcome for patients with Crohn's disease would aid in selection of appropriate therapy. This body of work aimed to define objective, longitudinal tools to improve prediction of outcome in Crohn's disease.

There were two critical features which defined our approach in this work. The first was the recording of objective clinical data in a longitudinal fashion. This was achieved by designing a database with dated datafields, and endeavoring to minimize subjectivity in datapoint recording. Further objective longitudinal data were obtained through data linkage to laboratory databases. The second feature was definition of an outcome which was reversible. This feature of outcome definition meant that analysis of longitudinal information was able to occur at many timepoints in a patient's disease course.

A poor outcome was defined as the formation of a bowel stenosis, perforation or fistula. Resolution of an outcome was defined as the passage of 2 years without further observation of the outcome. Resolution could occur following surgery, or passively with the passage of time. Perianal fistula formation was considered as an independent outcome in a separate analysis.

A consistently low albumin level $< 37\ \mathrm{g\,L^{-1}}$, a platelet count >370 x$10^9$/L, an MCV $< 86$ fL and a neutrophil count >8.6 x$10^9$/L were identified to be associated with subsequent bowel stenosis, fistula formation or perforation. Additionally, an albumin level consistently $< 38\ \mathrm{g\,L^{-1}}$ or a CRP consistently $> 11\ \mathrm{mg\,L^{-1}}$ were associated with subsequent perianal fistula formation. This information may lead to enhanced outcome prediction in Crohn's disease, and improved tailoring of therapy for individual patients. These findings require validation in an external cohort.

# Declaration by Author

This thesis is composed of my original work, and contains no material previously published or written by another person except where due reference has been made in the text. I have clearly stated the contribution by others to jointly-authored works that I have included in my thesis.

I have clearly stated the contribution of others to my thesis as a whole, including statistical assistance, survey design, data analysis, significant technical procedures, professional editorial advice, and any other original research work used or reported in my thesis. The content of my thesis is the result of work I have carried out since the commencement of my research higher degree candidature and does not include a substantial part of work that has been submitted to qualify for the award of any other degree or diploma in any university or other tertiary institution. I have clearly stated which parts of my thesis, if any, have been submitted to qualify for another award.

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# Publications during candidature

The following 5 abstracts have been accepted in poster format at Digestive Diseases Week 2015.

Consistently Low Albumin Level as a Predictor of Subsequent Bowel Stenosis, Fistula or Perforation in Patients with Crohn's Disease. *James Irwin, Emma Ferguson, Lisa A.Simms, Katherine Hanigan, Graham Radford-Smith* **DDW abstract number Su1277, DDW 2015, Washington DC May 16th - May 19th**

Consistently High C Reactive Protein is Associated with Subsequent Development of Perianal Fistulae in Patients with Crohn's Disease. *James Irwin, Emma Ferguson, Lisa A.Simms, Katherine Hanigan, Graham Radford-Smith* **DDW abstract number Sa1194, DDW 2015, Washington DC May 16th - May 19th**

A Novel Description of Longitudinal Phenotype in Patients with Crohn's Disease. *James Irwin, Emma Ferguson, Lisa A.Simms, Katherine Hanigan, Graham Radford-Smith* **DDW abstract number Su1335, DDW 2015, Washington DC May 16th - May 19th**

Delay in Meeting Formal Diagnostic Criteria in Crohn's Disease. *James Irwin, Emma Ferguson, Lisa A.Simms, Katherine Hanigan, Graham Radford-Smith* **DDW abstract number Su1196, DDW 2015, Washington DC May 16th - May 19th**

The Pre-diagnosis Inflammatory Signature of Patients with Inflammatory Bowel Disease. *James Irwin, Emma Ferguson, Lisa A.Simms, Katherine Hanigan, Graham Radford-Smith* **DDW abstract number Su1336, DDW 2015, Washington DC May 16th - May 19th**

## Publications Included in this thesis

None.

## Contributions by others to this thesis

This thesis is based on the body of work which precedes it. Graham Radford-Smith and the Inflammatory Bowel Diseases Group at the QIMR Berghofer have recruited patients into their research programme for the past 20 years and have performed serological and genetic testing on these patients. Without this preceding work this thesis would not have been possible.

Graham Radford-Smith, as my supervisor, has contributed significantly to the structure of this thesis and the chosen research directions. Many of the observations made by this thesis were developed in response to his ideas and opinions.

## Statement of parts of the thesis submitted to qualify for the award of another degree

No part of this thesis has been submitted to qualify for the award of another degree.

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## Keywords

inflammatory bowel disease, Crohn's disease, longitudinal data analysis, laboratory testing, outcome prediction

## Australia and New Zealand Standard Research Classifications

ANZSRC code:110307 Gastroenterology and Hepatology Clinical Sciences, 100%

## Fields of Research (FoR) Classification

FoR code:1103 Clinical Sciences, 100%

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# Abbreviations

| | |
|---|---|
| **ACTH** | **A**dreno**C**ortico**T**trophic **H**ormone |
| **ASCA** | **A**nti **S**accharomyces **C**erevisiae **A**ntibody |
| **ATG16L1** | **A**u**T**opha**G**y-related protein 16-L1 |
| **ATG5** | **A**u**T**opha**G**y-related protein 5 |
| five **ASA** | five **A**mino **S**alicylic **A**cid |
| **CDAI** | **C**rohn's **D**isease **A**ctivity **I**ndex |
| **CEACAM6** | **C**arcinoembryonic **A**ntigen-related **C**ell **A**dhesion **M**olecule 6 |
| **CRP** | **C** **R**eactive **P**rotein |
| **CT** | **C**omputed **T**omography |
| **DNA** | **D**eoxyribo**N**ucleic**A**cid |
| **EEN** | **E**xclusive **E**nteral **N**utrition |
| **ESR** | **E**rythrocyte **S**edimentation **R**ate |
| **GWAS** | **G**enome **W**ide **A**ssociation **S**tudy |
| **IBD** | **I**nflammatory **B**owel **D**isease |
| **IFN$\gamma$** | **I**nter**F**ero**N** $\gamma$ |
| **IL23R** | **I**nter**L**eukin 23 **R**eceptor |
| **IRGM** | **I**mmunity **R**elated **G**TPase family **M** protein |
| **JAK2** | **JA**nus **K**inase 2 |
| **6MMP** | 6 **M**ethyl**M**ercapto**P**urine |
| **MRI** | **M**agnetic **R**esonance **I**maging |
| **NHL** | **N**on **H**odgkins **L**ymphoma |
| **NF$\kappa\beta$** | **N**uclear **F**actor $\kappa\beta$ |
| **NOD2** | **N**ucleotide-binding **O**ligomerization **D**omain containing protein 2 |
| **OGD** | **O**esophago**G**astro**D**uodenoscopy |

| | |
|---|---|
| **QIMR** | **Q**ueensland **I**nstitute of **M**edical **R**research |
| **RBWH** | **R**oyal **B**risbane and **W**omen's **H**ospital |
| **PEN** | **P**artial **E**nteral **N**utrition |
| **PRDM1** | **PR** **D**o**M**ain containing Zinc Finger Protein 1 |
| **RNA** | **R**ibo**N**ucleic**A**cid |
| **SES-CD** | **S**imple **E**ndoscopic **S**core in **C**rohn's **D**isease |
| **SNP** | **S**ingle **N**ucleotide **P**olymorphism |
| **STAT** | **S**ignal **T**ransducer and **A**ctivator of **T**ranscription |
| 6**TGN** | 6 **T**hio**G**uanine **N**ucleotides |
| anti **TNF** antibody | anti **T**issue **N**ecrosis **F**actor alfa antibody |
| **USS** | **U**ltra**S**ound **S**can |

# 1. Literature Review

## 1.1. Crohn's disease

### 1.1.1. Biology of Crohn's disease

Crohn's disease is a chronic inflammatory condition which affects the human gastrointestinal tract. It was first described by Burrill Crohn *et al.* in 1932 as a chronic inflammatory condition affecting the terminal ileum, characterized by stenosis and fistula formation.(Crohn BB, Ginzburg L, and Oppenheimer GD 1932). The most commonly affected segment of bowel remains the terminal ileum, however in its current definition it may affect any part of the gastrointestinal tract, from the mouth to the anus. It is characterized histologically by chronic transmural inflammation of the gut wall, fissuring ulceration, transmural fibrosis, non-continuous involvement of bowel segments, and granuloma formation.(Lennard-Jones 1989)

The aetiology of Crohn's disease remains poorly understood.(Xavier and Podolsky 2007) It is hypothesized that an abberant chronic inflammatory state results from interplay between a susceptible innate and adaptive immune system, the barrier function of the intestinal wall and microbial gut flora.

Studies into the genetics of Crohn's disease have given insight into host dependent factors that contribute to the pathogenesis of Crohn's disease. The most strongly associated genes are discussed briefly in this section. NOD2 is an intracellular receptor protein expressed in immune and intestinal epithelial cells, especially Paneth cells. It contributes to an innate immune response to bacterial antigens through Nuclear Factor $\kappa\beta$ (NF-$\kappa\beta$) signaling.(Ogura et al. 2001) Mutations in the gene encoding this protein are associated with increased risk of developing Crohn's disease. ATG16L1 is a protein component of intracellular apparatus essential for autophagy.(Mizushima et al. 2003) Mutations in the ATG16L1 gene predispose to Crohn's disease, implying autophagy plays a role in gut defence. IL23R is a receptor protein expressed on the surface of immune cells, which increases cellular responsiveness to the cytokine interleukin 23.(Parham et al. 2002) It plays a role in the complex signalling between cells of the immune system that effects both an innate and adaptive immune response against foreign antigens. One mutation in the gene encoding IL23R confers protection against the development of Crohn's disease. JAK2 is a protein involved in the JAK/STAT signalling pathway that communicates signals (including interferon-$\gamma$ mediated signals) from receptors on the cell surface to the nucleus, altering gene expression.(Watling et al. 1993) Mutations in this gene are associated with uncontrolled cell proliferation, particularly the myeloproliferative disorders polycythaemia rubra vera and essential thrombocythaemia.(Baxter et al. 2005) They also are associated with the development of Crohn's disease, possibly due to an increase in intestinal permeability.(Prager et al. 2012) PRDM1 encodes a DNA binding protein which regulates transcription of the $\beta$-interferon gene and thus modulates immune function.(Keller and Maniatis 1991)

These genetic observations highlight the importance of the adaptive immune system in maintaining a healthy gut free of chronic inflammation. The intestinal microbial flora also play a role in perpetuating chronic Crohn's inflammation in the gut. Chronically inflamed mucosa represents a niche environment, with different survival pressures in comparison to gut mucosa with normal barrier function. This environment is rich in nutrients, but places pathogens in much closer proximity to

the host immune system. Some bacteria are able to adapt to this life. Adherent *Escherichia coli* are present in ileal mucosa affected by Crohn's disease in larger numbers than in unaffected mucosa.(Darfeuille-Michaud et al. 2004) Adherent *E. coli* from mucosa affected by Crohn's disease activate NF-$\kappa\beta$, reducing production of ATG16L1 and ATG5, and reducing effective autophagy.(H. T. T. Nguyen et al. 2014) Adherent *E. coli* bind to the mucosa of affected bowel by receptors for Carcinoembryonic Antigen-related Cell Adhesion Molecule 6 (CEACAM6).(Barnich et al. 2007) CEACAM6 expression on intestinal epithelial cells is upregulated by IFN-$\gamma$ and TNF-$\alpha$, cytokines produced by an active host immune system. These observations provide evidence that adherent *E. coli* have evolved an ability to modulate the host immune system to their survival advantage.

### 1.1.2. Epidemiology and natural history of Crohn's disease

The published prevalence of Crohn's disease varies from 50-200 per 100,000 people.(Gearry et al. 2006; Wilson et al. 2010; Loftus et al. 2007; Cosnes, Gower-Rousseau, et al. 2011) The peak age of onset is between 20-40 years of age, and it is more common in women than men by a factor of 1.3.(Gearry et al. 2006; Bernstein, Wajda, et al. 2006). Crohn's disease is more common in smokers.(Persson, Ahlbom, and Hellers 1990)

Most patients with Crohn's disease have involvement of the ileum only, the colon only, or of both the ileum and the colon. A small proportion of patients have isolated involvement of the upper gastrointestinal system (oesophagus, stomach and duodenum), or isolated orofacial involvement. Extent of disease remains stable in the majority of patients, although a minority extend to ileocolonic involvement from isolated colonic or ileal involvement, over their disease course.(Louis, Collard, et al. 2001)

The natural history of Crohn's disease is varied. The condition is chronic, and persists for most patients for decades. It is typically characterized by periods of disease activity separated by periods of remission.(Munkholm et al. 1995) Up to 30% of patients have a penetrating bowel complication (bowel perforation or fistula) or bowel stenosis at diagnosis.(Cosnes, S. Cattan, et al. 2002; Louis, Collard, et al. 2001) This proportion increases to 40%-50% at five years. Perianal fistulae are present in up to 20% of patients at diagnosis, and develop in a further 10% over the next five years.(Schwartz et al. 2002; Cosnes, S. Cattan, et al. 2002) Perianal fistulae occur more commonly in patients with colonic involvement of their luminal Crohn's disease.(D. R. Williams et al. 1981; Rankin et al. 1979; Veloso et al. 2001; Tang, Rawsthorne, and Bernstein 2006) Patients may also have symptomatic diarrhoea and abdominal pain on a chronic basis.

In the current environment between 25% - 50% of patients with Crohn's disease undergo bowel resection within 5 years of their diagnosis.(Ramadas et al. 2010) Malnutrition occurs in approximately 6% of patients with Crohn's disease. Fistulizing complications and bowel resection predispose to malnutrition.(G. C. Nguyen, Munsell, and Harris 2008) For those that go under extensive resection of their small bowel, there is a risk of short gut syndrome and consequent dehydration, electrolyte disturbance and malnutrition.(Fleming, McGill, and Berkner 1977)

Extra-intestinal manifestations are immune mediated inflammatory conditions which occur in patients with inflammatory bowel disease. They are: peripheral arthropathy, iritis or uveitis, ankylosing spondylitis, primary sclerosing cholangitis, erythema nodosum and pyoderma gangrenosum. They are more likely to be present when there is active luminal inflammation, and occur in up to 26% of patients with Crohn's disease.(Bernstein, Blanchard, et al. 2001; Rankin et al. 1979) The most commonly reported extraintestinal manifestation is peripheral arthropathy - a subjective symptom without an objective test to confirm diagnosis. When

peripheral arthropathy is excluded, prevalence of the remaining extraintestinal manifestations is around 6%.(Bernstein, Blanchard, et al. 2001).

## 1.2. Therapy in Crohn's disease

Immunosuppressive therapy, exclusive enteral nutrition, antibiotic therapy and surgery are currently used to treat Crohn's disease. These treatments alter patient symptomatology, and some have been demonstrated to alter disease course. Many of them have a significant adverse effect profile. The efficacy of currently used treatments, and their side effect profiles, are outlined in this section.

### 1.2.1. Medical therapy

Medical therapy in Crohn's disease is targeted at reducing inflammation, and preventing complications of chronic inflammation. The majority of effective therapies achieve this effect through modulation of the host immune system. Antibiotic therapy is aimed at modifying the composition of enteric bacterial populations. Exclusive enteral nutrition (EEN) is believed to reduce inflammation in Crohn's disease by either modifying the enteric bacterial population, and/or reducing the permeability of the gut wall.(Day and Burgess 2013)

The first medical therapy used in the treatment of Crohn's disease was Adrenocorticotrophic Hormone (ACTH) in 1951.(Stanley, Rosenberg, and Cleroux 1951) The effect of ACTH and cortisone in inducing clinical and biochemical remission was more clearly described over subsequent years.(Cooke and Fielding 1970) The side effect profile of prolonged steroid use led to the use of steroid sparing immunosuppression for maintenance of remission.(Sandborn, Sutherland, et al. 1996; Pearson, May, G. Fick, et al. 2000)

Medical therapy for Crohn's disease is considered in two contexts. Firstly, to return a patient to health while acutely unwell with active Crohn's inflammation - *remission induction.* Secondly, to maintain health in a patient who is well without active Crohn's inflammation - *maintenance of remission.*(Dignass et al. 2010)

*Steroid therapy*

Corticosteroids are the medication most commonly used to induce remission in active Crohn's disease. They are associated with a relative risk of 1.99 of remission induction when compared to placebo.(Benchimol et al. 2008) They reduce bowel inflammation through a number of different mechanisms. They effect a reduction in gene transcription of inflammatory cytokines including TNF-$\alpha$ and IL-6.(Yang and Lichtenstein 2002) They also inhibit the effect of NF$\kappa\beta$ on transcription of non-steroid dependent inflammatory cytokines.(Yang and Lichtenstein 2002)

*Thiopurines*

Currently azathioprine and 6 mercaptopurine are commonly used in the management of Crohn's disease. These are prodrugs which are metabolized by the liver to their active metabolites 6 thioguanine nucleotides (6TGN). 6TGN are thiopurine analogues which interrupt purine synthesis, DNA and RNA synthesis, and ultimately cell replication.(Sandborn, Sutherland, et al. 1996) Both drugs induce remission of active luminal Crohn's disease (OR=2.36, NNT=5), and are associated with maintenance of remission (OR=2.32, NNT=6).(Sandborn, Sutherland, et al. 1996; Pearson, May, G. Fick, et al. 2000; Prefontaine et al. 2009) In a meta analysis, thiopurine use was associated with resolution of perianal fistulae.(Pearson, May, G. H. Fick, et al. 1995) However, this effect has not been demonstrated as a primary endpoint in a controlled trial.

*Methotrexate*

Methotrexate is a folinic acid analogue and inhibits cellular metabolism. It has been shown to induce remission (OR=1.95) and maintain remission (OR=1.67) in luminal Crohn's disease.(Feagan, Rochon, et al. 1995; Feagan, Fedorak, et al. 2000)

*Anti TNF-$\alpha$ inhibition*

TNF-$\alpha$ is an inflammatory cytokine which plays an important role in the process which drives inflammation in Crohn's disease. The anti TNF-$\alpha$ inhibitors infliximab, adalimumab and certolizumab are manufactured antibodies with affinity for TNF-$\alpha$. They have been shown to be effective in inducing and maintaining remission in Crohn's disease.(J. F. Colombel et al. 2010; J.-F. Colombel et al. 2007; Schreiber et al. 2007) Infliximab and adalimumab have been shown to aid healing of perianal fistulae.(Present et al. 1999; J.-F. Colombel et al. 2007) In these two trials closure of fistulae at 1 year occurred in 30-40% of patients in the treatment arms compared with 10% in placebo arms.

Combination therapy using infliximab and a thiopurine medication has been shown to be more effective than therapy with either medication alone at inducing remission in patients with Crohn's disease.(J. F. Colombel et al. 2010) This is postulated to be due to either a synergistic immunosuppressive effect, or to a reduction in formation of anti-infliximab antibodies in the presence of thiopurine immunosuppression.

*Anti-integrins*

$\alpha_4\beta_1$ and $\alpha_4\beta_7$ integrins are molecules which regulate adherence and migration of lymphocytes from the vascular system into brain and gut tissue respectively. Natalizumab inhibits both of these molecules and has been shown to induce and maintain remission in Crohn's disease.(Sandborn, J. F. Colombel, et al. 2005; Targan et al. 2007) Vedolizumab inhibits $\alpha_4\beta_7$ integrin only and also induces and maintains remission in Crohn's disease.(Sandborn, Feagan, et al. 2013)

*Antibiotic therapy*

Metronidazole has been shown to reduce postoperative recurrence of Crohn's disease. (Rutgeerts et al. 1995) Antibiotics are used in conjunction with surgical drainage to control perianal sepsis. Ciprofloxacin given for 12 weeks in combination with adalimumab increased the rate of healing of perianal fistulae.(Dewint et al. 2014) This response lost clinical significance at 24 weeks.

*Exclusive Enteral Nutrition*

ENN is the provision of all nutritional requirements through a polymeric enteral feed or an elemental (amino acid, glucose and fat) enteral feed, and the exclusion of all other oral intake.(Day and Burgess 2013) EEN is effective at inducing remission in paediatric Crohn's disease.(Day and Burgess 2013; Johnson et al. 2006) In partial enteral nutrition (PEN) a proportion of the nutritional requirements of the patient is delivered via an enteral feeding tube, while a normal diet is taken orally. Remission induction occurs with EEN and not PEN, suggesting that the efficacy of the therapy depends on the removal of normal food from the diet.(Johnson et al. 2006)

### 1.2.2. Adverse effects of medical therapy

*Infection*

Use of steroid, thiopurine, or anti TNF-$\alpha$ therapy in Crohn's disease is associated with an increased risk of infection.(Glazier et al. 2005; Lichtenstein, Feagan, et al. 2012; Klein, Go, and Cunha 2001) The most commonly reported infections are pneumonia, cellulitis, urinary tract infection and abscess formation.(Lichtenstein, Feagan, et al. 2012; Glazier et al. 2005) Absolute rates of significant infection in patients with Crohn's disease are of the order of 1-2 per 100 patient years.(Lichtenstein, Feagan, et al. 2012) Data regarding risk of infection associated with immunosuppression in Crohn's disease are confounded by higher rates of immunosuppression use in sicker patients, who have increased risk of infection independent of immunosuppression use.(Epple 2009)

Opportunistic infections cause disease in immunocompromised hosts, while in immunocompetent hosts they cause mild illness only. Anti TNF-$\alpha$ medications, corticosteroids and thiopurines increase risk of opportunistic infection.(Lichtenstein, Feagan, et al. 2012; Toruner et al. 2008) The most clinically relevant of these is the reactivation of latent tuberculosis.(Raval et al. 2007) Reactivation of latent tuberculosis in the setting of anti TNF-$\alpha$ therapy is associated with use of a second immunosuppressive drug (methotrexate, azathioprine, prednisone).(Raval et al. 2007)

In case studies anti TNF-$\alpha$ medications have been reported to cause reactivation of hepatitis B.(Esteve et al. 2004)

*Hepatitis*

Drug induced hepatitis can result from thiopurine, methotrexate or anti TNF-$\alpha$ therapy.(Derijks et al. 2006; Feagan, Rochon, et al. 1995; Moum et al. 2007; Menghini and Arora 2001) Harm from drug induced hepatitis may be reduced by routine blood testing identifying asymptomatic biochemical hepatitis, leading to cessation of therapy before hepatitis becomes clinically apparent. Thiopurine induced hepatitis is associated with blood 6 methyl mercaptopurine (6MMP) levels $>$5700 pmol/$10^8$ erythrocytes.(Dubinsky et al. 2000) Risk of significant thiopurine associated hepatitis may also be minimized by monitoring thiopurine metabolites and dose adjusting when indicated.

*Cytopenia*

Thiopurine, methotrexate and anti TNF-$\alpha$ use is associated with cytopenia.(Markowitz 2003; Salar et al. 2007; Lim, Gaffney, and D. G. I. Scott 2005) Blood count monitoring while on therapy may reduce the risk of severe cytopenia, and of consequent bleeding and infective complications.

*Infusion reaction or injection site reaction*

6% of patients have a reaction to infused infliximab, and approximately 1% of these are severe reactions characterized by hypotension, shortness of breath, tachycardia

and facial flushing.(Cheifetz et al. 2003). Non-severe reactions can be managed by slowing of infusion rate and administration of hydrocortisone. Injection site reactions occur in 3% of patients using adalimumab or certolizumab.(J.-F. Colombel et al. 2007; Schreiber et al. 2007)

*Hypersensitivity reaction*

Thiopurines carry a 2% risk of a hypersensitivity reaction after initiation.(Sandborn, Sutherland, et al. 1996) This reaction is characterized by fever, arthralgia, myalgia, rash and raised inflammatory markers. It is typically symptomatically severe and necessitates cessation of the drug. $^2/_3$ of patients suffering a hypersensitivity reaction to azathioprine are able to tolerate subsequent introduction of 6 mercaptopurine.(Nagy et al. 2008)

*Anti TNF-$\alpha$ induced Systemic Lupus Erythmatosis (SLE)*

Administration of anti TNF-$\alpha$ medication stimulates cell lysis and exposure of DNA to the host immune system. An immune response to these antigens can occur, leading to the development of drug induced SLE.(Vermeire, Noman, et al. 2003) Incidence of drug induced lupus is of the order of 0.2% of treated patients.(De Bandt et al. 2005) The condition resolves with cessation of the drug.

*Skin cancer*

In Australia the risk of non-melanoma skin cancer is of the order of 1170 per 100,000 person years.(Staples et al. 2006) For those less than 40 the risk is 210 per 100,000 person years, while for those of over the age of 70 the risk is 7305 per 100,000 person years. (Staples et al. 2006) Incidence of non-melanoma skin cancer is increased by a factor of 6 in patients taking thiopurines.(Peyrin-Biroulet et al. 2011) There are no clear data to suggest that anti TNF-$\alpha$ medications are associated with non-melanoma skin cancer.(Lichtenstein, Feagan, et al. 2012) Melanoma appears to be more common in patients with inflammatory bowel disease. There has been no association between immunosuppressive medication use

and melanoma. (Singh et al. 2014) There are conflicting data regarding the risk of melanoma associated with the use of anti TNF-$\alpha$ medications.(Singh et al. 2014; Long et al. 2012)

*Lymphoma*

Incidence of Non-Hodgkin's Lymphoma (NHL) in the general population increases from 1 per 10,000 person years for those aged less than 40, to 9 per 10,000 person years for those aged over 80.(Kandiel et al. 2005). Risk of NHL is probably not increased in patients with Crohn's disease.(Lewis et al. 2001) Thiopurine use increases the risk by a factor of 4, and combination thiopurine and anti TNF-$\alpha$ use increases the risk by a factor of 6.(Siegel et al. 2009; Kandiel et al. 2005)

There have been 36 reports of hepatosplenic T-cell lymphoma in patients taking either thiopurine monotherapy or combined anti TNF-$\alpha$ and thiopurine immunosuppression for inflammatory bowel disease.(Kotlyar et al. 2011) This is a cancer which is often fatal and has predominantly affected men under the age of 35. It is difficult to assess incidence from what are a collection of reported adverse events, however for men under the age of 35 it has been estimated to be 1/7404 for those taking thiopurine monotherapy, and 1/3534 for those taking an anti TNF-$\alpha$ agent and a thiopurine in combination.(Kotlyar et al. 2011)

*Progressive Multifocal Leukoencephalopathy*

Progressive Multifocal Leukoencephalopathy is a progressive neurological condition. It is an opportunistic infection caused by reactivation of latent JC (John Cunningham) polyomavirus, and has been observed in 2/1000 patients treated with the anti-integrin antibody natalizumab in post marketing analysis. 20% of those affected die, and half of survivors are left with severe neurological disability.(Bloomgren et al. 2012) Vedolizumab is not associated with this condition.(Sandborn, Feagan, et al. 2013)

### 1.2.3. Surgical therapy

Surgery plays a major role in the management of Crohn's disease. It can be performed to relieve chronic symptoms which are refractory to medical therapy. For example, resection of a symptomatic stenosis, or colectomy in colitis refractory to medical therapy. It also is considered early in the course of disease, with the goal of providing a prolonged period of good health, without medical therapy and its associated side effects.(Latella, Caprilli, and Travis 2011) Surgery plays an important role in the management of perianal fistulae. Finally, Crohn's disease can lead to acute severe complications which require emergency surgery, such as acute bowel perforation or obstruction. However, the majority of surgery performed in Crohn's disease is elective.(Siassi et al. 2007)

*Small bowel or colonic resection*

Bowel resection in Crohn's disease removes diseased segments of bowel, and is the operation of choice for ileal and colonic Crohn's disease. Bowel segments may become severely damaged from chronic inflammation, with little likelihood of medical therapy repairing the functionality of the damaged tissue and relieving symptoms. Symptomatic bowel stenosis, chronic enterocutaneous fistulae, enterovesical fistulae or enterovaginal fistulae are examples of such situations.(T. Yamamoto and Watanabe 2014) Chronic inflammatory intra-abdominal or retroperitoneal collections, which remain in communication with an involved segment of bowel, cause chronic inflammation and pain. They are considered an indication for resection of the involved bowel. Finally, resection of the colon is performed in chronic refractory colitis.

Surgery in Crohn's disease aims to remove complications of chronic inflammation (stricture, fistula) without removing all diseased bowel.(Alexander-Williams and Haynes 1987) This approach acknowledges that extensive removal of bowel does not cure Crohn's disease, and increases risk of short gut syndrome.

*Colonic resection*

Surgical options for colonic Crohn's disease include proctocolectomy with permanent end ileostomy formation, colectomy and ileorectal anastamosis, or limited colonic resection. Disease recurrence in the rectum is a common outcome after ileorectal anastamosis. However, severe symptoms leading to permanent ileostomy formation were observed to occur in 14% of patients in the 10 years following reanastamosis.(P. Cattan et al. 2002) Ileal pouch-anal anastamosis is associated with a significant rate of fistulizing complications and is usually avoided in Crohn's disease.(T. Yamamoto and Watanabe 2014)

*Stricturoplasty*

In diffuse jejunal disease, or in cases of multiple previous bowel resections, there is appreciable risk of short gut syndrome with further bowel resection. Stricturoplasty is considered a valid bowel preserving operation to relieve obstructive symptoms. In one series the rate of recurrent stricture at the site of stricturoplasty was of the order of 20-30% at 6 years.(Dietz et al. 2002)

*Surgical management of perianal fistulae*

Perianal fistulae expose perianal tissues to faecal contamination, and are a source of recurrent localized sepsis. Surgical management of perianal fistulae, performed in combination with medical therapy, increase the likelihood of their resolution, and reduce the severity of symptoms suffered by patients.

Prompt surgical drainage of perianal fistulae is required when they occur. Insertion of a non-cutting seton provides drainage and reduces incidence of recurrent abscess formation.(Gecse et al. 2013) There is no consensus on optimal timing for removal of setons in perianal Crohn's fistulae. Risk of recurrent abscess increases

with removal of the seton, however healing of the fistula will not occur until the seton is removed.

Low perianal fistulae which do not involve the sphincter muscle complex are amenable to laying open without risk of loss of continence. Healing of fistulae with this procedure are of the order of 80%.(H. J. Scott and Northover 1996)

There is no clear evidence regarding the efficacy of rectal flap advancement for the treatment of high perianal fistulae in Crohn's disease.(Soltani and Kaiser 2010) Observational studies cite wide ranges of success of this procedure (33-92%). It is accepted to be less successful for rectovaginal fistulae.(Soltani and Kaiser 2010)

Formation of a loop ileostomy diverts the faecal stream from the colon and is performed to increase the likelihood of healing of severe perianal Crohn's disease. The ostomy may be reversed after a period of time, although recurrent disease precludes reversal for the majority of these patients.(T. Yamamoto, Allan, and M. R. Keighley 2000)

In cases of severe perianal Crohn's disease proctectomy and permanent ileostomy relieves symptoms of chronic debilitating perianal Crohn's disease.(Mueller et al. 2007)

### 1.2.4. Adverse effects of surgery

Surgery entails risk of complications such as anastomotic breakdown, intra-abdominal sepsis, formation of enterocutaneous fistulae through the surgical wound, wound infection, pulmonary embolism or pneumonia.(D. T. Yamamoto, Allan, and M. R. B. Keighley 2000) The risk varies depending on a number of factors, including the

surgical procedure being performed, whether it is an acute or elective procedure, steroid use leading up to the operation, patient age, nutritional status and the presence of obesity. The risk of a significant complication is in the order of 13%.(D. T. Yamamoto, Allan, and M. R. B. Keighley 2000)

## 1.3. Decision making in the management of Crohn's disease

The management of Crohn's disease is a long term undertaking. Physicians advise patients to take therapies with a significant side effect profile for many years, with the expectation of reducing current symptoms and reducing the risk of future complications of their disease. This complex decision making is dependent on assessment of risk of adverse outcome in the absence of therapy, the risks of therapy, and of improvement in outcome attributable to the chosen therapy.

The management of patients with Crohn's disease represents a significant cost to healthcare organizations.(Bernstein, Longobardi, et al. 2012) The largest direct costs are hospital admission and anti TNF-$\alpha$ prescription. In addition to the direct cost of providing treatment, indirect costs due to loss of work productivity are also incurred. Anti TNF-$\alpha$ medications have significantly increased the cost of therapy in Crohn's disease. In Australia the annual cost of anti TNF-$\alpha$ therapy is of the order of $AU30,000 - $AU40,000 per patient per year.(Australian Government Department of Health 2014a; Australian Government Department of Health 2014b). This expense is likely to be offset by direct and indirect savings due to reduced hospital admissions and increased work productivity.(Bernstein, Longobardi, et al. 2012)

The provision of all expensive pharmaceuticals available today to those who would benefit from them would be likely to bankrupt any public health system. Expensive therapies such as anti TNF-$\alpha$ medications currently need to be rationally allocated by a public health system if their use is to be sustainable. In today's environment, cost has some influence over choice of therapy for individual patients.

### 1.3.1. Utility of outcome prediction

Outcome prediction in Crohn's disease allows physicians to tailor an individual patient's therapy based on their likely disease course. A high likelihood of poor outcome will increase the potential benefit of aggressive therapy, and make it more attractive despite its side effect profile.

## 1.4. Poor outcome in Crohn's disease

### 1.4.1. Outcome definition

The model of Crohn's disease on which current treatment strategy is based is of chronic bowel inflammation leading to bowel damage, and symptoms resulting from either tissue damage or from inflammation itself.(Cosnes, S. Cattan, et al. 2002) It may take many years for evidence of tissue damage to become apparent.(Thia et al. 2010; Cosnes, S. Cattan, et al. 2002) The advancement of therapeutic options to manage Crohn's disease has depended on timely assessment of efficacy of novel therapies. Rather than waiting many years for development of tissue damage, efficacy has been measured by change in *disease activity* attributable to the therapy being tested.(for example; J. F. Colombel et al. 2010)

Degree of bowel inflammation, or severity of symptoms attributable to Crohn's disease, are both used as measures of disease activity. The gold standard measurement of bowel inflammation is considered to be endoscopic assessment. However, the invasive nature of colonoscopy means that clinical symptoms, or non-invasive biological markers, are often used to measure disease activity. Validated measures of short-term outcome include the Simple Endoscopic Score in Crohn's Disease (SES-CD, see table 1), mucosal healing (defined as the absence of ulceration at ileocolonoscopy), the Crohn's Disease Activity Index (CDAI, see table 2), or biological markers such as C-Reactive Protein (CRP) and faecal calprotectin. (Daperno et al. 2004; De Cruz et al. 2013; Best et al. 1976; Schoepfer, Beglinger, et al. 2010) The most commonly used clinical measurement of disease activity in clinical trials is the CDAI. This index sums scores of bowel frequency, patient reported severity of abdominal pain, general wellbeing, presence of perianal disease, presence of extraintestinal manifestations of inflammatory bowel disease (arthralgia, iritis, erythema nodosum, apthous ulceration, pyoderma gangrenosum), fever, use of antidiarrhoeal medications, haematocrit and weight. Disease activity - as measured by CDAI, endoscopic assessment or with biological markers - is often used as an endpoint in pharmacological trials, as a measure of response to immunomodulatory therapy.(for example; J. F. Colombel et al. 2010) These are the outcome variables which have been used to demonstrate the efficacy of the medical treatments listed in section 1.2.1.

Chronic disease activity is associated with symptoms which reduce quality of life, and is considered to increase the risk of surgery.(Lichtenstein, Yan, et al. 2004) Of all measures of disease activity, absence of mucosal healing is the measure that has been most strongly predictive of surgery in Crohn's disease.(Schnitzler et al. 2009)

### 1.4.2. Definition of long term outcome

Crohn's disease is a chronic condition. Descriptions of long term outcome allow clinicians to inform newly diagnosed patients of how Crohn's disease may affect their life. Validated measures of disease activity are not measures of long term outcome. Long term outcome is typically measured by major events in the natural history of Crohn's disease. Examples used in longitudinal studies include bowel resection, a requirement for long term steroid use and a requirement for immunosuppressive therapy.(Ramadas et al. 2010; Solberg et al. 2007)

Clear definitions of poor long term outcome allow observation of occurrence of such an outcome in a cohort, and also observation of which patients tend to progress to the outcome. This information could be used to stratify patients with Crohn's disease into those likely to benefit from aggressive therapy, and those whose disease course may not warrant the side effect profile.

In the literature there is variability in the definition of a poor long term outcome in Crohn's disease.(Beaugerie and Sokol 2012) The term *disabling disease* was coined by Beaugerie et al. to describe a poor clinical course.(Beaugerie, Seksik, et al. 2006) It was defined as one or more of the following: administration of two or more courses of steroids, long term steroid use, hospitalization for a disease flare, presence of disabling chronic symptoms (cumulative time of more than 12 months of disabling symptoms (diarrhea with nocturnal and/or urgent stools, intense abdominal pain because of intestinal obstruction, fever, fatigue attributable to the disease, joint pain, painful uveitis or pyoderma gangrenosum), a need for immunosuppressive therapy, undergoing an intestinal resection, or undergoing a perianal surgical procedure. This definition was also used in a validation study.(Loly, Belaiche, and Louis 2008) In the study by Loly *et al.* a second analysis was performed which defined *severe Crohn's disease* as one or more of the following: the development of complex perianal disease, any colonic resection, two

or more ileal resections, a single resection of 50cm or more of small bowel, or creation of a permanent stoma.

Pariente *et al.* have proposed an objective scoring system, the *Lémann Score*, to describe outcome in Crohn's disease.(Pariente, Cosnes, et al. 2011; Pariente, Mary, et al. 2015, see table 5). This score is being developed with the goal of standardizing the measurement of poor outcome in Crohn's disease. It assigns a score to represent tissue damage at each of 4 bowel segments: the upper gastrointestinal tract, the small bowel, the colon, and the perineum/anus. Tissue damage may be measured on clinical examination, at ileocolonoscopy, at oesophagogastroduodenoscopy (OGD), on radiological imaging, at surgery or on examination of surgical specimens. The score is cumulative, meaning once tissue damage has been observed at one segment it is never considered normal again.

### 1.4.3. Difficulties in long term outcome definition

Many of the outcome variables used to define poor outcome in the literature to date lack objectivity. Measures such as *a need for immunotherapy*, or *hospitalization for a disease flare* are subject to variable physician threshold. Symptoms such as fatigue, joint pain or abdominal pain are by nature subjective and will be reported variably by patients. A lack of objectivity means that results from studies using these outcome definitions are difficult to compare to each other. Additionally, it is difficult for physicians to determine how such results will translate to their own patient population.

### 1.4.4. Predictors of currently defined poor outcome

Three clinical variables have been identified as predictive of *disabling disease* as defined by Beaugerie *et al*: Steroid use at diagnosis, age below 40 years at diagnosis and perianal disease at diagnosis.(Beaugerie, Seksik, et al. 2006) Stricturing

disease at diagnosis and weight loss greater than 5kg at diagnosis predict the development of *severe Crohn's disease* as defined by Loly *et al.* Currently these are the variables which are taken into consideration by most physicians when making decisions regarding therapy for patients with Crohn's disease.

Further variables have been noted to correlate with poor outcome in Crohn's disease. NOD2 genotype is associated with the development of stenotic or penetrating complications(Adler et al. 2011), active smoking with penetrating complications (Louis, Michel, et al. 2003), and anti *Saccaromyces cerevisiae* antibody (ASCA) positivity with small bowel stenosis. (Vasiliauskas et al. 2000; Forcione et al. 2004)

# 2. Hypotheses

## 2.1. Hypothesis generation

### 2.1.1. Measurement of outcome

Our initial literature review covered prediction of outcome in Crohn's disease. Initial consideration focused on identifying novel predictors of outcome in Crohn's disease, or significant unidentified interactions between recognized predictors of outcome. Genome wide association studies (GWAS) have identified 140 single nucleotide polymorphisms (SNPs) associated with Crohn's disease, when comparing cases with non-affected controls.(Jostins et al. 2012; Hugot et al. 2001; Franke et al. 2010; Barrett et al. 2008) An interaction between ATG16L1 and smoking increasing risk of Crohn's disease has been reported,(Fowler et al. 2008) and other interactions are likely to exist. A significant proportion of the RBWH Crohn's cohort has been genotyped, and so exploration of the interaction between genetic and environmental variables and outcome was strongly considered.

Having identified genetic variants associated with incidence of Crohn's disease, the literature has progressed to explore their relationship with Crohn's phenotype. NOD2 variants have been associated with ileal disease location, earlier onset of disease, penetrating and stricturing phenotype (Cleynen et al. 2012; Weersma et al. 2009; Economou et al. 2004; Jung et al. 2012), while ATG16L1 variants have been associated with ileal location of disease.(Fowler et al. 2008) IL23R genotype and JAK2 genotype are associated with colonic location of disease.(Cleynen et al.

2012) JAK2 is associated with stenosing phenotype, and PRDM1, NOD2 variants and IL23R are associated with penetrating phenotype.(Cleynen et al. 2012) The number of patients in our cohort (~300) meant that genotype/phenotype analysis was not likely to be a successful strategy due to lack of statistical power and was not pursued further.

During this literature review it became apparent that definitions used to describe outcome in Crohn's disease vary, and additionally often describe outcome using subjective variables.(Ramadas et al. 2010; Beaugerie, Seksik, et al. 2006; Loly, Belaiche, and Louis 2008) To illustrate this point, an outcome variable repeatedly used in the Crohn's literature is a requirement for surgery. Surgery is performed for patients with Crohn's disease based on physician and surgeon opinion of its benefit, and patient agreement to undergo the proposed procedure. In some cases this is a clear cut decision (for example in acute peritonitis from bowel perforation), however in other situations opinion between clinicians varies (for example early ileocolic resection for isolated inflammatory terminal ileal Crohn's disease).(Latella, Caprilli, and Travis 2011) Additionally, patients may refuse to undergo surgery. Although a major event in a patient's disease course, the performance of surgery per se. is unlikely to represent a reproducible and objective measure of disease progression. In support of this observation, there have been significant differences in rates of surgery observed across centres in Europe.(Wolters, M. G. Russel, et al. 2006)

The International Program to develop New Indexes in Crohn's disease (IPNIC) is developing a system to objectively classify bowel damage in Crohn's disease - the *Lémann* score.(Pariente, Cosnes, et al. 2011; Pariente, Mary, et al. 2015) It derives a score calculated from perforation, stricture formation or performance of surgery, for each of four segments of the human gastrointestinal tract - the colon, the small bowel, the upper gastrointestinal tract, and the anus/perianal region. (See table 5). The pertinent features of the *Lémann* score are that it is reproducible, objective, and measures bowel damage irrespective of whether or not surgery is

performed. This score shows promise in providing an objective and reproducible measure of outcome in Crohn's disease.

### 2.1.2. Longitudinal predictor variables

Our cohort of Crohn's disease patients have had a large number of objective tests performed on them as part of their clinical management. As far as we are aware, these type of data have not been used significantly in the Crohn's disease literature to date.(Vermeire, Van Assche, and Rutgeerts 2006) Laboratory data in particular are measured in a serial fashion, and represent an opportunity to consider prediction of outcome at many different timepoints in a patient's disease course. We have recognized these data as a silo of objective longitudinal information describing our patients, and have incorporated them into our analysis.

## 2.2. Statement of hypotheses

1. That collection of objective, longitudinal clinical data will allow further insight into the natural history of Crohn's disease, beyond that provided by traditional phenotyping by Montreal or Vienna classification systems.

2. That a set of objective, reproducible and clinically relevant measures of poor outcome in Crohn's disease may be defined *for use in longitudinal analyses.*

3. That objective variables, measurable in a longitudinal fashion throughout a patient's disease course, will aid in predicting progression to a poor outcome in Crohn's disease.

# 3. Methodology

## 3.1. Definition of poor outcome

We have defined poor outcome in Crohn's disease for use in longitudinal analysis (Table 3.1). This definition has been carefully considered to represent measurable, objective tissue damage with significant symptomatic consequences. It has also been defined to allow multiple occurrences of an endpoint during a patient's disease course.

This is a noteworthy point of difference from the Montreal classification of disease behaviour and the *Lémann* score. (Satsangi et al. 2006; Pariente, Mary, et al. 2015) Both of these scores consider disease complication or outcome in a cumulative fashion, and therefore a patient may never reduce their score over time. This means that both of these systems by definition will observe worsening disease over time. They are also less able to analyze further disease progression following assignment of a high score. To illustrate this point consider possible disease courses following an ileocolic resection for terminal ileal stricture or perforation. One patient may have 3 further anastomotic resections over the subsequent 10 years for recurrent stricture, and a second patient may have no further complication. The Montreal behaviour classification does not distinguish between these two patients despite their markedly different clinical course. We feel that it is crucial that our outcome measure is able to distinguish between such patients. Our approach to achieving this is to define outcome in a way that allows resolution of an observed outcome.

## 3.2. Data collection

### 3.2.1. Datapoint selection

Objective data were entered into a database designed to record a longitudinal description of disease course for patients with Crohn's disease. Data was obtained from the clinical record, and from the RBWH phenotypic research database. Datapoints were selected according to the following criteria:

1. Include previously described predictors of poor outcome in Crohn's disease: steroid use at diagnosis, perianal disease at diagnosis, age at diagnosis, stricturing behaviour at diagnosis, weight loss at diagnosis and platelet count at diagnosis. (Loly, Belaiche, and Louis 2008; Beaugerie, Seksik, et al. 2006)
2. Include longitudinal laboratory data.
3. Include outcome variables as described in table 3.1: radiology results, endoscopy results, operative surgical and histological findings, clinical observation of enterocutaneous fistulae, findings at examination under anaesthetic (EUA), and perianal examination findings.
4. Include variables considered to have an influence over outcome: medication use, smoking.

A critical aspect of how these data have been coded is their temporal nature. For example, the use of thiopurine immunosuppression has not been recorded as "yes, >6 months" or "no". Instead this information has been coded using datapoints "date start", "date stop", "dose","cessation reason" for each thiopurine used. This allows detailed temporal association of recorded variables with outcome.

A coding system for selected datapoints was defined and reviewed with Graham Radford-Smith. This system was then used to code a pilot group of 15 patients, which identified datapoint omissions and coding inconsistencies. These were addressed and optimized before embarking on coding the entire cohort. The attached document *Longitudinal Crohn's Database Coding Manual* describes in detail the datapoints and how they have been coded.

### 3.2.2. Included variables

Table 3.2 lists the variables included in this analysis.

| *Objective endpoint* | *Modality of observation* | *Criteria* |
|---|---|---|
| Stricture development | Imaging | Bowel lumen narrowing *and* proximal dilatation $\geq$ 2.5cm |
| | Surgery | Stricture identified by surgeon or pathologist |
| | Endoscopy | Stricture identified by endoscopist, unable to be traversed |
| Perforation | Imaging | Extraluminal collection *or* free air in the abdomen *or* phlegmon |
| | Surgery | Extraluminal collection *or* phlegmon *or* faecal contamination of abdomen |
| Fistula | Imaging | Radiologically evident fistula |
| | Surgery | Surgical evidence of fistula |
| | Clinical examination | Evidence of entero-cutaneous fistula *or* entero-vesical fistula |
| Perianal disease | Imaging | Radiologically evident perianal fistula |
| | Surgery | Evidence of perianal fistula |
| | Clinical exam | Evidence of perianal fistula |

**Table 3.1**
Definition of poor outcome in Crohn's disease.
*1: Development of a single endpoint represents a poor outcome.*
*2: Timing of endpoint development is at its earliest detection.*
*3: Endpoints which result from surgery or endoscopic procedures are excluded.*
*4: Endpoints are considered present until they are resolved. Resolution is defined as the passage of two years without further observation of the endpoint. Endpoints may resolve with surgery, or passively.*
*5: Endpoints which occur before resolution of a previous endpoint are considered part of that endpoint.*
*6: Resolution of an endpoint will return a patient to the status of "no endpoint met".*
*7: Intra-abdominal and perianal endpoints are considered separately.*
*Imaging includes MR enterography, CT enterography, USS, barium meal, barium enema, anal ultrasound.*
*Endoscopy includes ileocolonoscopy, flexible sigmoidoscopy, OGD, enteroscopy, capsule endoscopy.*

| **Predictor Variables** | **Outcome Variables** |
|---|---|
| *Laboratory Values* | *Endoscopy Data* ⋆ |
| Haemoglobin | Colonoscopy |
| White cell count | Flexible sigmoidoscopy |
| Neutrophil count | OGD |
| Platelet count | Capsule endoscopy |
| CRP | Enteroscopy |
| ESR | |
| Ferritin | *Radiology Data* ⋆ |
| Transferrin saturation | MR enterography |
| Faecal calprotectin | CT enterography |
| Albumin | USS |
| ASCA | Barium meal |
| ANCA | Barium enema |
| 6TG metabolites | Pelvic MRI |
| | Anal USS |
| *Clinical Variables* | |
| Appendicectomy | |
| Family History of IBD | *Surgical Data* ⋆ |
| Pregnancy | Laparoscopy/laparotomy |
| Age | Perianal surgery |
| Ethnicity | |
| Year of Diagnosis | *Clinical Examination* ⋆ |
| Smoking | Perianal fistula |
| Sex | Enterocutaneous fistula |
| Clinic attendance | |
| Weight | *Other Outcomes* |
| | Hospital admission |
| *Medication Use* | Intravenous steroid use |
| Five ASA | Long term steroid use ($>$ 6 continuous months) |
| Thiopurine | |
| Methotrexate | |
| Anti-TNF antibody | |
| Anti-integrin antibody | |
| Oral steroid | |
| Intravenous steroid | |
| *Genetic Variables* | |
| NOD2 | |
| ATG16L1 | |
| IL23R | |

**Table 3.2**
Collected variables.
⋆ *Data collected from endoscopy procedures, imaging, surgery, and clinical examination are used to identify luminal or perianal fistulae, perforation, intra-abdominal or perianal abscess formation or bowel stenosis.*

## 3.3. Cohort selection

### 3.3.1. Royal Brisbane and Women's Hospital Crohn's disease cohort

The RBWH IBD unit came into being with the arrival of Graham Radford-Smith to the hospital in the early 1990's. Before this time patients with IBD were managed by many gastroenterologists in the hospital. Graham focused his clinical work in the field of IBD and has maintained an active research program. Under his leadership an inflammatory bowel disease unit has evolved which provides all IBD care at RBWH. He and the unit have been recruiting patients with inflammatory bowel disease into the RBWH and QIMR Berghofer research programme from 1994.

The cohort of patients with Crohn's disease which are studied in this thesis are those that have been treated at the RBWH IBD unit, and have been consented for the research programme by Graham and his team over this time.

### 3.3.2. Inclusion and exclusion criteria

To be included in the cohort patients need to meet the following inclusion and exclusion criteria:

1. Inclusion Criteria
    (a) Meet the *Lennard-Jones* criteria for a diagnosis of Crohn's disease (Lennard-Jones 1989) - see table 3.
    (b) Have a minimum of five years of follow-up in the clinical record.
2. Exclusion Criteria
    (a) Diagnosis before 1994.

(b) Lack of longitudinal laboratory data.

There is no clear consensus regarding diagnostic criteria for Crohn's disease. The most commonly cited diagnostic criteria are the Lennard-Jones criteria. (Lennard-Jones 1989) These criteria depend on demonstrating evidence of transmural disease involvement (submucosal fibrosis, submucosal ulceration, evidence of perforation, fistula or macroscopic fibrosis). Because of this a diagnosis is often not formally met until surgery has been performed and a macroscopic surgical specimen is obtained for analysis. This concern has been acknowledged by the European Crohn's and Colitis Organization (ECCO) who formed a consensus statement which covers the diagnosis of Crohn's disease. (Van Assche et al. 2010) The ECCO diagnostic criteria are less strict, however are not clearly defined. This is illustrated by the following two quotes from this document. (Figures 3.1, 3.2)

> A single gold standard for the diagnosis of CD is not available. The diagnosis is confirmed by clinical evaluation and a combination of endoscopic, histological, radiological, and/or biochemical investigations. Genetic testing is currently not recommended for routine diagnosis or management of CD. [EL5, RG D].

**Figure 3.1**
Consensus statement 2B from ECCO consensus guidelines, pg 12.
(Van Assche et al. 2010)

> The current view is that the diagnosis is established by a non strictly defined combination of clinical presentation, endoscopic appearance, radiology, histology, surgical findings and, more recently, serology. This still results in diagnostic obstacles.

**Figure 3.2**
Taken from text of ECCO consensus guidelines, pg 12.
(Van Assche et al. 2010)

Therefore diagnostic criteria for this cohort were *consistent* with the Lennard-Jones criteria. However, patients who did not undergo surgery, and therefore did not have a transmural surgical specimen for examination, were not required

to demonstrate evidence of transmural bowel involvement. For these patients evidence of chronic mucosal bowel inflammation in a typical distribution (ileal only, or non-continuous colonic) was considered adequate for diagnosis.

## 3.4. Data collection

### 3.4.1. Database design

The database is designed in SQLite. It is in a simple table format, with all datapoints on one row and one row per patient. Data analysis was performed with the data in a relational format. An algorithm (written in R) converts the data into a relational format for subsequent analysis.(See coding definition 3)

### 3.4.2. Data linkage

All patients who have consented to take part in the RBWH IBD research programme, or are clinical patients treated by the RBWH IBD department, are assigned a unique identifying number. This number identifies all data attributable to that patient on a clinical and phenotypic database. Genetic and serological analysis performed on patient blood and serum at the QIMR Berghofer is also recorded against this number. Data linkage between patients and genetic data has been performed using this identification number.

The Queensland public health system does not maintain a single statewide medical record number, and individual patients may have multiple different identification numbers which vary by attended public hospital. For this reason data linkage to laboratory data stored on the AUSLAB and PARIS databases has been made using identifying data which were common between databases: *first-name / last-name / date of birth / sex*. The same matching data was used to link our

cohort to data from S&N (Sullivan and Nicoloides) and QML (Queensland Medical Laboratories) databases. The pertinent features of the databases to which we have linked data are outlined below.

1. AUSLAB database
    (a) Queensland wide, public health system laboratory data
    (b) Tests ordered on our patients at other public hospitals in Queensland captured
    (c) Electronic recording of results began in 1999
2. PARIS database
    (a) Greater Brisbane region, public health system laboratory data
    (b) Tests ordered on our patients at other public hospitals in Greater Brisbane captured
    (c) Electronic recording of results 1985 - 1998
3. S&N database
    (a) A private laboratory service
    (b) Electronic record began in 2001
4. QML database
    (a) A private laboratory service
    (b) Electronic record began in 1995
5. QIMR Berghofer IBD research group genetic database
    (a) Matched to Crohn's disease cohort by unique identifying number

## 3.5. Statistical analysis

### 3.5.1. Cohort size

Our cohort size was determined by the number of patients who met our inclusion criteria, and is currently 302 patients with a median of 10 years of follow-up. A calculation of statistical power would not have altered our approach beyond deciding whether or not it was likely to be worthwhile collecting the data and performing the analysis, as there was no capacity to increase the size of the cohort. We estimated that the size of the cohort would allow identification of significant association between predictor and outcome variables using Cox regression, including up to 10 predictive variables in a final model.

The power of this cohort to detect association between predictor and outcome variables is dependent on the frequency of occurrence of outcome variables, and the distribution of predictor variable status amongst patients. We estimated the frequency of outcome over 10 years to be in the order of 30-50%, based on published figures for the development of penetrating or stricturing complications of Crohn's disease.(Thia et al. 2010)

### 3.5.2. Statistical approach

Recording longitudinal data allowed temporal association of predictor and outcome variables. Correlation between predictor variables and outcome was performed allowing study subjects to experience multiple outcome events as described in the method by Prentice et al.(Prentice, B. J. Williams, and Peterson 1981) (see figure 3.3). This method analyzes predictor variables as a function of time to event

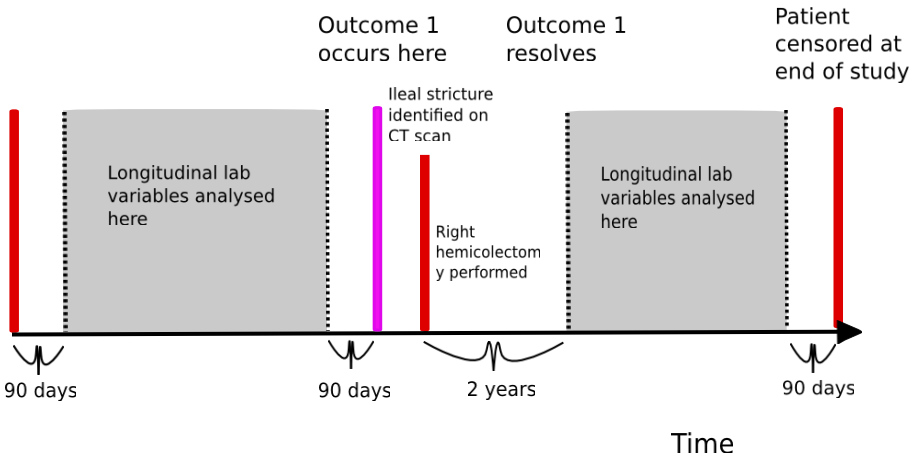


**Figure 3.3**
Strategy for analysis of longitudinal data

from date of diagnosis *or* from resolution of the immediately preceding outcome, whichever was the more recent.

Temporal laboratory variables used as predictive variables were analyzed in relation to the outcome variable using Cox regression analysis. For each variable a variation on area under the curve was used to convert multiple values into a single representative value. (see code definition .1.5)

In the final model continuous variables were converted to dichotomous categorical variables, which we consider are more easily interpretable by clinicians. This was performed using an automated algorithm which maximized association between a predictor variable and outcome depending on the cutoff used. This was performed using the log-rank statistic and univariate Cox regression. (see code definition 15)

# 4. Description of Cohort and Objective Data Collected

Review of records at the Royal Brisbane and Women's Hospital IBD unit and at the QIMR Berghofer identified 360 patients diagnosed with Crohn's disease after the 1st January 1994 and before 31st March 2008. 58 of these had less than 5 years of clinical follow-up and 11 had inadequate laboratory data available for analysis.(table 4.1).

| | |
|---|---|
| Total | 360 |
| <5 years follow-up | 58 |
| Inadequate lab data | 11 |
| Included | 291 |

**Table 4.1**
Excluded patients

The method of data recording for each event is demonstrated by figure 4.2, which shows as an example how a single colonoscopy was recorded.

| **Field** | **Entry** |
|---|---|
| id | 766.001 |
| ColoDate | 1995-08-02 |
| ColoInvestigation | 1 |
| ColoInd | 2 |
| ColoExtent | 6,7,8,9,10,11 |
| ColoFist | 0 |
| ColoStenosis | 6 |
| ColoSevereEndoLesions | 0 |
| ColoIntervention | 0 |
| ColoHistoMicro | F |
| ColoHistoMicroCoded | 3,1,2,4 |
| ColoHistoMicroExtent | 6,8,9 |

**Table 4.2**
Example of coding for a colonoscopy

# 5. Consistently Abnormal Laboratory Results Predict Subsequent Bowel Stenosis, Fistulization or Perforation in Patients with Crohn's Disease.

## 5.1. Authors

James Irwin, (1,2,3) Emma Ferguson, (2) Lisa A Simms, (3) Katherine Hanigan, (3) Graham Radford-Smith (1,2, 3)

## 5.2. Institutions

1. Department of Gastroenterology and Hepatology, Royal Brisbane and Women's Hospital, Brisbane, Australia.

2. School of Medicine, The University of Queensland, Brisbane, Australia.

3. Inflammatory Bowel Diseases Research Group, QIMR Berghofer Medical Research Institute, Brisbane, Australia

## Summary Box

What is already known:

Outcome prediction in Crohn's disease helps tailor therapy to individual patients. The following clinical features predict worse outcome and should lower threshold for increasing intensity of treatment.

- Age < 16 years at diagnosis
- Receiving steroids for first disease flare
- Presence of perianal disease at diagnosis

What are the new findings:

Consistently low albumin $< 38 \mathrm{g\,L^{-1}}$, high platelet count $> 370\text{x}10^9$/L, low mean cell volume < 86 fL and high neutrophil count $> 8.7\ \text{x}10^9$/L are associated with subsequent bowel stenosis, fistulization or perforation.

How might it impact on clinical practice in the foreseeable future:

If validated in other cohorts, these laboratory variables could be used in addition to recognized clinical features to enhance prediction of poor outcome in Crohn's disease, and to tailor therapy more accurately to those who are most likely to benefit from it.

## 5.3. Abstract

*Objective:* To study the correlation between longitudinal laboratory testing and subsequent bowel stenosis, bowel perforation or intra-abdominal fistula formation in patients with Crohn's disease.

*Design:* Patients diagnosed at a tertiary referral centre with Crohn's disease between 1st January 1994 and 31st March 2008, with more than five years of follow-up, were analyzed. An objective poor outcome (OPO) was defined as the development of a fistula, bowel stenosis or bowel perforation. Laboratory data were recorded when patients were well, prior to each OPO. Cox regression was used to analyze the association between OPO development and; C reactive protein, erythrocyte sedimentation rate, platelet count, haemoglobin level, mean cell volume, white

blood cell count, neutrophil count, albumin, alanine transaminase, vitamin D, vitamin B12, folate, faecal calprotectin, serum iron, transferrin saturation and ferritin level.

*Results:* In 291 patients observed over a median of 10.49 years, blood testing was performed a median of 4.72 (IQR 3.07-7.12) times per year, over a median of 4.79 (IQR 2.84–7.76) years prior to each OPO. 183 OPOs (116 independent stenoses, 14 independent perforations, 15 independent fistulae and 38 combination events) were observed. After multivariate analysis an albumin level consistently $< 37$ $g\,L^{-1}$ (HR 4.41, p<0.001), a platelet count $> 370$ $x10^9/L$ (HR 2.22, p=0.037), an MCV $< 86$ fL (HR 2.71, p=0.002) and a neutrophil count $> 8.6$ $x10^9/L$ (HR 5.39, p=0.002) maintained independent association with OPO. L1 Montreal location at diagnosis (HR 2.80, p=0.005) and having suffered a previous OPO (HR 3.08, p<0.001) were also independently associated with OPO in the final model.

*Conclusion:* Consistently abnormal albumin level, platelet count, MCV and neutrophil count are associated with subsequent OPO development in patients with Crohn's disease. These tests may signal risk of development of subsequent OPO, and may provide a rationale for escalation of therapy. These findings require validation in an external cohort.

## 5.4. Introduction

Crohn's disease is a chronic inflammatory condition of the human gastrointestinal tract of undetermined aetiology. It is characterized by a relapsing and remitting course (Munkholm et al. 1995) and by significant morbidity from chronic abdominal pain, diarrhoea, perianal abscess and fistula formation, bowel stenosis and obstruction, internal fistulization and bowel perforation.(Lazarev et al. 2010; Cosnes, Nion-Larmurier, et al. 2005; Ramadas et al. 2010; Tarrant et al. 2008; Thia et al. 2010; Magro et al. 2014)

Treatment with corticosteroids, the thiopurines azathioprine and 6-mercaptopurine, methotrexate, the anti tissue necrosis factor (TNF) alpha inhibitors infliximab and adalimumab and the anti-integrins natalizumab and vedolizumab have been demonstrated to reduce evidence of active Crohn's disease when measured by the Crohn's disease activity index (CDAI) and by endoscopic assessment of bowel mucosa. (Markowitz 2003; Pearson, May, G. Fick, et al. 2000; Feagan, Rochon, et al. 1995; Feagan, Fedorak, et al. 2000; J. F. Colombel et al. 2010; J.-F. Colombel et al. 2007; Sandborn, J. F. Colombel, et al. 2005; Sandborn, Feagan, et al. 2013; Benchimol et al. 2008) There is observational evidence that anti TNF alfa therapy is associated with a reduction in rates of intestinal surgery, (Feagan, Panaccione, et al. 2008) and conflicting evidence that azathioprine and 6-mercaptopurine are associated with a reduction in rates of intestinal surgery, or rates of stenotic or penetrating complications. (Ramadas et al. 2010; Cosnes, Nion-Larmurier, et al. 2005; Feagan, Panaccione, et al. 2008; Chatu et al. 2014; Lakatos et al. 2012) Immunomodulatory therapy in Crohn's disease carries a significant side effect profile. For patients without symptoms from their Crohn's disease, a decision to embark on long term immunomodulatory therapy rests on assessment of risk of development of a poor long term outcome, and the magnitude of expected reduction in this risk associated with medication use.

Features of a patient's clinical presentation and early disease course allow some prediction of the likelihood of a subsequent poor clinical course. This allows clinicians scope to limit immunomodulatory medication exposure to patients most likely to benefit from them. Age <16 at diagnosis, perianal disease at diagnosis, and requirement for corticosteroids to control the first flare of disease are published, validated and widely used predictors of a poor outcome described as disabling disease.(Beaugerie, Seksik, et al. 2006; Loly, Belaiche, and Louis 2008) Additionally there are published data demonstrating an association between NOD2 genotype and development of stenotic or penetrating complications (Adler et al. 2011), active

smoking with penetrating complications,(Louis, Michel, et al. 2003) Anti *Saccharomyces cerevisiae* antibody (ASCA) positivity with small bowel stenosis (Vasiliauskas et al. 2000; Forcione et al. 2004) and raised CRP and platelet count at diagnosis with subsequent bowel resection. (Boirivant et al. 1988; Loly, Belaiche, and Louis 2008; Henriksen et al. 2008)

Current predictors of poor outcome suffer from low discriminatory power in identifying patients likely to follow a complicated disease course, and from non-uniformity of outcome measure. Further stratification of patients into those more and less likely to follow a poor clinical course would allow improved tailoring of immunomodulatory therapy, and improved management.

Published literature associating laboratory testing and long term outcome in Crohn's disease demonstrate an association between both a high CRP and a high platelet count at diagnosis, with subsequent intestinal surgery.(Loly, Belaiche, and Louis 2008; Henriksen et al. 2008) Additionally an association has been observed between haemoglobin level and subsequent surgery or disease complication. (Rieder et al. 2014) There are little published data analyzing the association between longitudinal laboratory testing and subsequent outcome. Longitudinal laboratory testing is a routine part of the management of patients with Crohn's disease. Testing is performed to assess inflammatory status, nutritional status, and to monitor for side effects from prescribed medications. These results represent a silo of objective clinical information which may provide useful prediction of future disease course. We hypothesize that these results contain a measurable signal that is associated with active and progressive Crohn's disease, and that this signal is associated with the subsequent development of an objective poor outcome (OPO).

## 5.5. Objective

To study the correlation between longitudinal laboratory testing and subsequent development of bowel stenosis, bowel perforation or intra-abdominal fistula formation in patients with Crohn's disease.

## 5.6. Patients and methods

This was a single centre observational longitudinal cohort study. All patients with a diagnosis of Crohn's disease made between 1$^{st}$ January 1994 and 31$^{st}$ March 2008, managed at the Inflammatory Bowel Diseases Unit at the Royal Brisbane and Women's Hospital (RBWH, Brisbane, Australia), were invited to participate in the research programme. Serological, epidemiological, clinical and genetic data were obtained for each patient. Further clinical data was obtained through retrospective review of the clinical record.

Ethical approval for the study was obtained through the Royal Brisbane and Women's Hospital ethics committee. All participating patients consented to take part in the research programme.

### 5.6.1. Definitions

The date of diagnosis was defined as the first date when the patient was felt to meet diagnostic criteria for Crohn's disease (Lennard-Jones 1989) by the treating physician. This was usually when tissue was first obtained at either colonoscopy or surgery, confirming chronic bowel inflammation in the setting of chronic abdominal symptoms. An OPO was defined as the first observation of an intestinal fistula, stenosis or perforation. This observation could be made at surgery, on macroscopic examination of a surgical specimen, at colonoscopy or gastroscopy, on computed tomography (CT) scanning, on magnetic resonance imagining (MRI),

or in the case of enterocutaneous fistulae on clinical examination. Perianal fistulae were not considered an OPO. An OPO was considered resolved 2 years following the time it was last observed. Resolution could result from surgery, or from the passage of time. The observation period prior to the development of an OPO in which laboratory data were examined was defined as follows: Ninety days following date of diagnosis, ninety days prior to first identification of the OPO, ninety days prior to censoring at end of study period if no OPO was identified in the observation period, and if the observation period followed a previous OPO, following resolution of the previous OPO (Figure 5.1). Laboratory variables taken within 90 days of abdominal surgery were excluded. These temporal exclusions were set to minimize the influence of two possible sources of bias. The first possible bias is that the presence of an occult, undiagnosed OPO, either at diagnosis or in the months leading up to OPO, would lead this analysis to misinterpret an association between occult OPO presence and subsequent OPO identification as an association between laboratory variables taken in the absence of an OPO and subsequent OPO development. The second possible bias is that an association between abdominal surgery and abnormal laboratory values, and an association between abdominal surgery and subsequent OPO development, would be misinterpreted as an association between abnormal laboratory values and subsequent OPO development.

### 5.6.2. Inclusion and exclusion criteria

All included patients met criteria for a diagnosis of Crohn's disease.(Lennard-Jones 1989) These were consistent with the Lennard-Jones criteria, however patients who did not undergo surgery, and therefore did not have a transmural surgical specimen for examination, were not required to demonstrate evidence of transmural bowel involvement. For these patients evidence of chronic mucosal bowel inflammation in a typical distribution (ileal only, or non-continuous colonic) was considered adequate for diagnosis. All early clinical information was reviewed to confirm that diagnostic criteria were met. Patients who did not have 5 years of follow-up

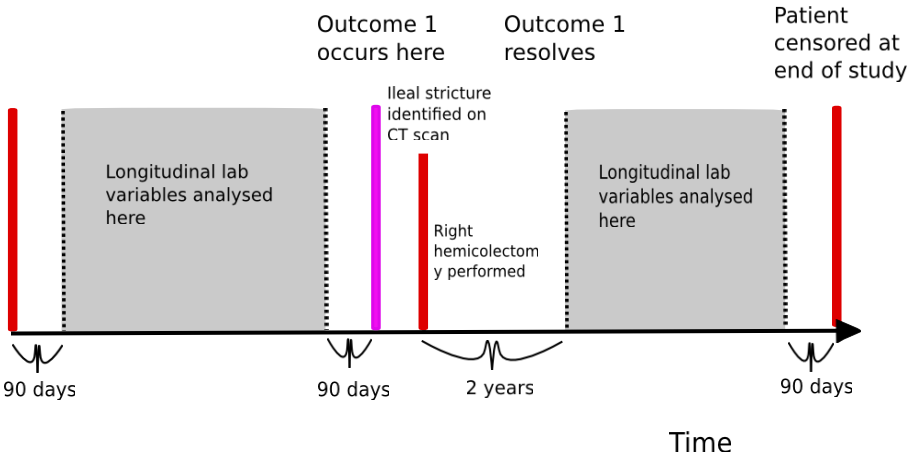


**Figure 5.1**
Cartoon demonstrating observation period from which lab data are taken. This patient contributes two observation periods to the analysis: the first leading up to identification of an ileal stricture on CT scan, the second when he/she reaches the end of the study period without suffering a second OPO. The second observation period does not begin until 2 years have passed without observation of the previous ileal stricture.

data available were not included in the analysis. OPO events which had less than two laboratory variables recorded in the immediately preceding observation period were excluded. OPO events which had an observation period of less than 180 days were excluded.

### 5.6.3. Laboratory data

Laboratory data were obtained by matching identities to data from four sources: AUSLAB (all laboratory results performed in public hospitals across Queensland 1$^{st}$ Jan 1999 to present), PARIS (historical database which records all laboratory results performed in public hospitals in Brisbane 1$^{st}$ Jan 1985 - 1$^{st}$ Jan 1999), Queensland Medical Laboratories (QML, private laboratory database covering all of Queensland 1$^{st}$ Jan 1995 to present) Sullivan and Nicholaides Pathology (SNP, private laboratory database covering all of Queensland 1$^{st}$ Jan 2001 – present). QML and SNP are the two major private pathology providers in Queensland. Patients were matched by surname, first-name, date of birth (DOB) and sex.

$$lvAUC = \sum_{1}^{n-1} (time_{x+1} - time_x) \times \left( \min(value_x, value_{x+1}) + \frac{|value_{x+1} - value_x|}{5} \right)$$

**Figure 5.2**
Area under curve calculation: for variables that usually rise with inflammation or malnutrition (platelet count, CRP, ESR, WBC count, neutrophil count, faecal calprotectin, ALT)
min = minimum
max = maximum

$$lvAUC = \sum_{1}^{n-1} (time_{x+1} - time_x) \times \left( \max(value_x, value_{x+1}) - \frac{|value_{x+1} - value_x|}{5} \right)$$

**Figure 5.3**
Area under curve calculation: for variables that usually fall with inflammation or malnutrition (albumin, haemoglobin, ferritin, serum iron, MCV, transferrin saturation, serum folate, red cell folate, vitamin D, vitamin B12)

C reactive protein (CRP, $\mathrm{mg\,L^{-1}}$), erythrocyte sedimentation rate (ESR, mm/h), haemoglobin level ($\mathrm{g\,L^{-1}}$), white blood cell count (WBC, $\mathrm{x10^9/L}$), platelet count ($\mathrm{x10^9/L}$), neutrophil count ($\mathrm{x10^9/L}$), mean cell volume (MCV, fL), faecal calprotectin ($\mu\mathrm{g\,g^{-1}}$ faeces), albumin ($\mathrm{g\,L^{-1}}$), alanine transaminase (ALT, U/L), serum ferritin ($\mathrm{mg\,L^{-1}}$), serum iron ($\mu\mathrm{mol\,L^{-1}}$), transferrin saturation (%), vitamin D level ($\mathrm{nmol\,L^{-1}}$), red cell folate ($\mathrm{nmol\,L^{-1}}$), serum folate ($\mathrm{nmol\,L^{-1}}$) and vitamin B12 level ($\mathrm{pmol\,L^{-1}}$) were analyzed. When analyzed as continuous variables, values reported as below the lowest detectable level were considered equal to zero, while all values reported above a highest detectable level were considered equal to that highest detectable level. These considerations were most important for CRP which had a lower limit of detection of 5 $\mathrm{mg\,L^{-1}}$ in the 1990's, reducing to 2 $\mathrm{mg\,L^{-1}}$ in 2006.

A representative value (laboratory variable Area Under Curve, lvAUC) for each laboratory variable, for each observation period, was calculated using a variation of the Area Under the Curve (AUC) using all eligible dated laboratory values ordered temporally ($\mathrm{value_n}$ taken at $\mathrm{time_n}$, see figures 5.2, 5.3).

### 5.6.4. Statistical analysis

Statistical analysis was performed in the R statistical computing environment. (R Core Team 2014) Correlation between lvAUC values variables and OPO was analyzed using Cox regression, allowing study subjects to experience multiple outcome events as described in the method by Prentice et al.(Prentice, B. J. Williams, and Peterson 1981, see Figure 5.1). In short, predictor variables were analyzed as a function of time to event from date of diagnosis or from resolution of the immediately preceding OPO, whichever was the most recent 5.1. In the exploratory analysis, lvAUC for each variable observed over the immediately preceding observation period was correlated with OPO using univariate Cox regression. lvAUC variables with a p-value of association $<0.2$ were then entered into a multivariate model in a stepwise fashion, retaining those with independent correlation ($p<0.05$) in a multivariate model. To make the analysis more clinically relevant, lvAUC variables were converted to dichotomous categorical variables, with optimized cut-off values determined using an automated algorithm. These relationships were adjusted for potential confounding by including the following variables in a final multivariate model: age at diagnosis, sex, date of diagnosis, Montreal classification disease location at diagnosis,(Silverberg et al. 2005) perianal disease at diagnosis, smoking status at diagnosis, having experienced a previous OPO, ATG16L1 genotype, NOD2 genotype, IL23R genotype, and use of intravenous steroids for first disease flare. Bias introduced through relatedness between repeated OPO events observed in the same patient was controlled for by including the variable *experienced previous OPO* in the multivariate model.

## 5.7. Results

291 patients contributed data for analysis.(Figure 5.4) Demographics are shown in Table 5.1. 309 OPO events were observed over a median follow-up period of 10.49 years. These comprised 179 independent stenoses, 30 independent fistulae, 35 independent perforations and 65 combination stenosis and perforation/fistula

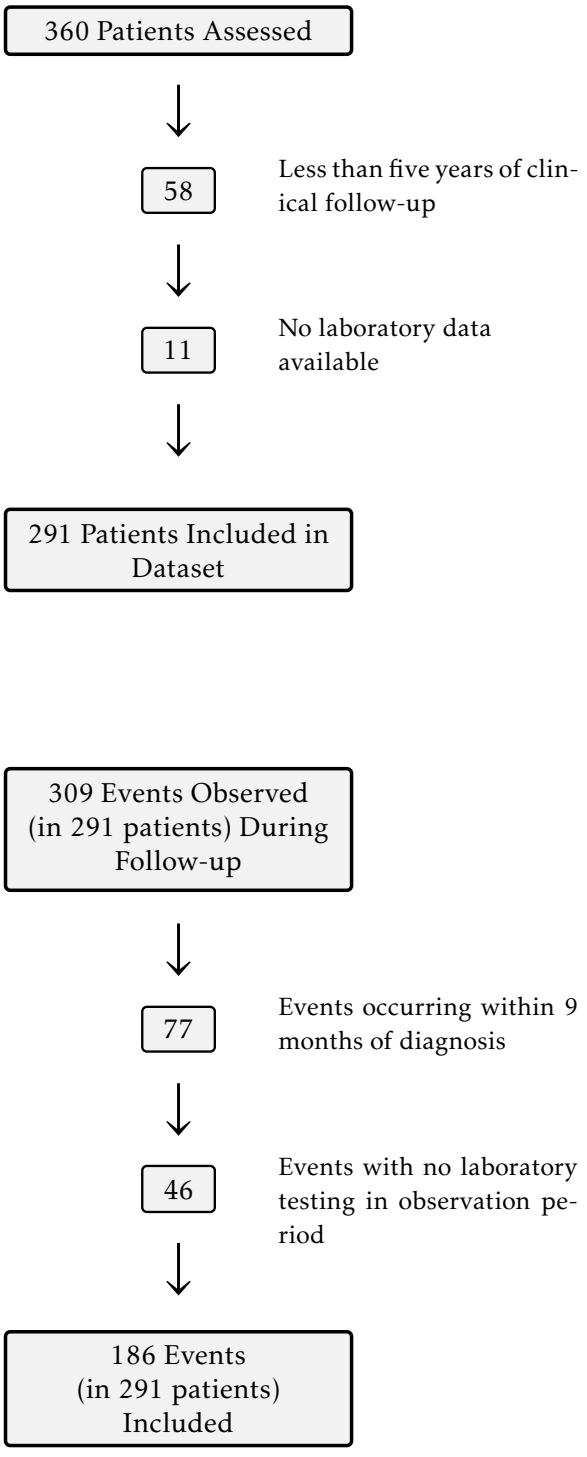


**Figure 5.4**
Exclusions.

events (combination events). 45 stenoses, 18 perforations, 8 fistulae and 16 combination events occurred within 9 months of diagnosis and were excluded. 18 stenoses, 3 perforations, 7 fistulae and 11 combination events occurred without laboratory data in the preceding observation period and were therefore excluded. 116 independent stenoses, 14 independent perforations, 15 independent fistulae and 38 combination events contributed to the analysis. 162 patients suffered no OPO event over the observation period, 87 patients suffered one, 32 suffered 2, 8 suffered 3, and 2 suffered 4. The median number of laboratory results for each test over each observation period are tabulated in Table 5.2. Data for faecal calprotectin were available for less than 1/3 of all observation periods, partly because faecal calprotectin was only used routinely to aid management after 2008.

| Demographics | |
|---|---|
| Age (mean, years) | 27.3 |
| Female | 55.33 % |
| Caucasian Australian | 86.25 % |
| Smoker at Diagnosis | 50.17 % |
| Family History IBD | 27.49 % |
| Follow-up (median, years) | 10.49 |
| | |
| Montreal Location at Diagnosis | |
| L1 | 124 |
| L2 | 57 |
| L3 | 106 |
| No macroscopic ileocolonic disease | 4 |

**Table 5.1**
Demographics.

| | Platelet Count | Haemoglobin | Albumin Level | CRP | Faecal Calprotectin |
|---|---|---|---|---|---|
| Proportion of observation periods with results | 1 | 1 | 1 | 0.97 | 0.3 |
| Median number of results per observation period | 21 (11-36) | 21 (11-36) | 18 (10-32) | 15 (8-27) | 4 (2-6) |
| Median tests per year | 4.72 (3.07-7.12) | 4.73 (3.11-7.27) | 4.33 (2.78-6.5) | 3.8 (2.45-5.92) | 1.88 (1.2-2.68) |

**Table 5.2**
Number of laboratory tests (prior to development of OPO, or prior to censuring at study end), used to calculate lvAUC value for each observation period.
Number of OPO or censored events = 418.

lvAUC values for all analyzed laboratory variables are shown in Table 5.4, stratified by whether the observation period ended in an OPO or in censoring. Figure 5.5 demonstrates the relationship between raw albumin results and the lvAUC value over one observation period for one patient. Correlation between continuous lvAUC laboratory values and OPO are tabulated in Table 5.4. Correlation between possible confounding variables and OPO are tabulated in Table 5.5. Having suffered a previous OPO, and L1 Montreal location at diagnosis, were both positively correlated. There was a trend for younger age to be correlated with OPO, while presence of perianal disease at diagnosis and IV steroid use at diagnosis were not correlated. Optimal cutoff values for conversion of continuous to categorical laboratory variables are shown in Table 5.6. A final multivariate model of independent variables is tabulated in Table 5.7. Table 5.8 shows the sensitivity, specificity, positive and negative predictive value of these features in prediction of development of OPO when applied to the derivation cohort.

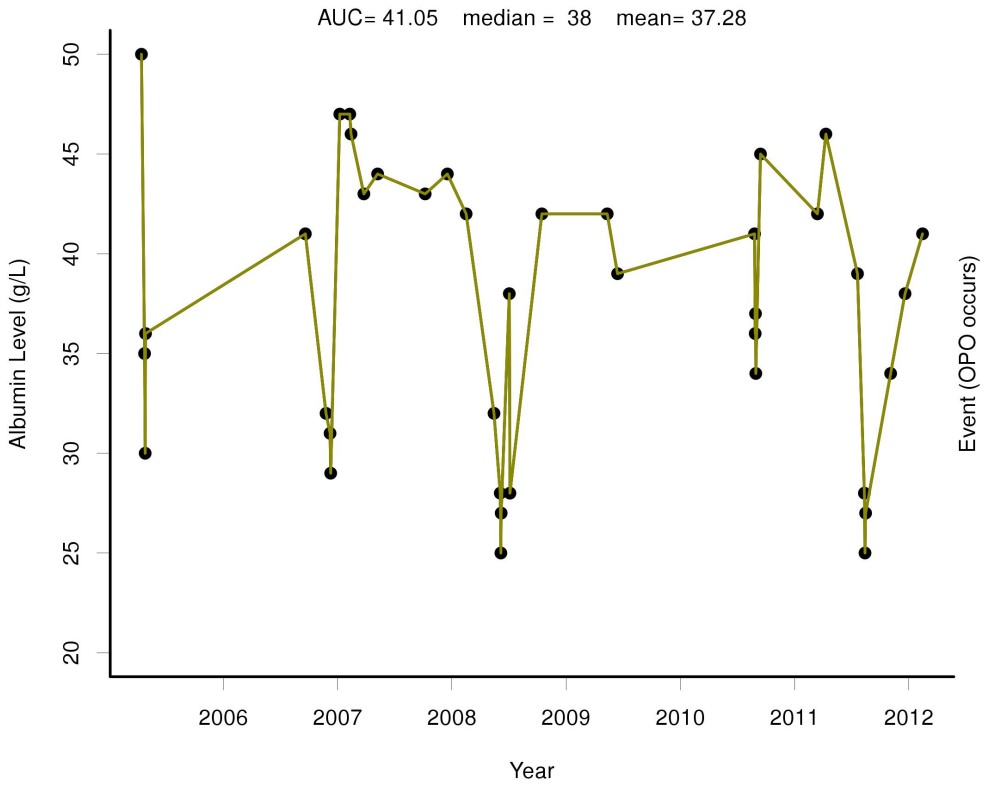


**Figure 5.5**
Derivation of the lvAUC value for a single sequence of albumin results.

| Variable | OPO | No OPO | p-value (t-test) |
|---|---|---|---|
| Haemogobin ($g\,L^{-1}$) | 131.48 (123.91 - 140.22) | 135.65 (127.38 - 144.77) | 0.003* |
| MCV (fL) | 89 (85.2 - 93.02) | 90.34 (87.84 - 94.59) | 0.005* |
| Platelet count ($x10^9$/L) | 299.39 (259.26 - 361.02) | 274.03 (237.56 - 317.88) | <0.001 ** |
| White Cell Count ($x10^9$/L) | 7.71 (6.32 - 9.32) | 6.94 (5.93 - 8.17) | <0.001 ** |
| Neutrophil Count ($x10^9$/L) | 5.12 (4.13 - 6.33) | 4.39 (3.56 - 5.43) | <0.001 ** |
| Albumin ($g\,L^{-1}$) | 40.01 (37.32 - 41.68) | 41.56 (39.61 - 43.5) | <0.001 ** |
| ALT (U/L) | 15.77 (11.21 - 20.21) | 17.97 (13.83 - 25.58) | <0.001 ** |
| CRP ($mg\,L^{-1}$) | 8.65 (3.97 - 16.61) | 5.04 (1.72 - 10.46) | <0.001 ** |
| ESR (mm/h) | 15.28 (8.73 - 23.57) | 11.99 (7.24 - 21.38) | 0.073 |
| Calprotectin ($\mu g\,g^{-1}$) | 200.39 (54.62 - 341.09) | 141.7 (60 - 402.8) | 0.766 |
| Ferritin ($\mu g\,L^{-1}$) | 63.65 (38.4 - 100) | 77.61 (42.7 - 127.15) | 0.041* |
| Serum Iron ($\mu mol\,L^{-1}$) | 11.9 (7.97 - 15.21) | 14.61 (11.23 - 18.14) | <0.001 ** |
| Transferrin Saturation (%) | 18.64 (13.6 - 26.37) | 22.98 (16.81 - 28.12) | 0.005* |
| Vitamin D ($nmol\,L^{-1}$) | 80.7 (69 - 104.8) | 79.09 (66.12 - 99.27) | 0.52 |
| Serum Folate ($nmol\,L^{-1}$) | 30.62 (12.92 - 34.72) | 32.78 (28.6 - 38.9) | 0.154 |
| Red Cell Folate ($nmol\,L^{-1}$) | 1045.9 (788.4 - 1369.58) | 1106.75 (857.9 - 1441.86) | 0.162 |
| B12 ($pmol\,L^{-1}$) | 388.48 (294.31 - 504.18) | 324.18 (244 - 426.78) | 0.015* |

**Table 5.3**
Comparison of lvAUC values over observation periods prior to OPO events, with lvAUC values over observation periods prior to censoring.
lvAUC values reported as Median (Lower Quartile - Upper Quartile)
p-value is for a two tailed t-test comparing the difference of two means.

| | $\beta$ coefficient | Hazard Ratio | p-value † |
|---|---|---|---|
| Haemogobin | -0.019 | 0.98 | 0.002 ⋆ |
| MCV | -0.038 | 0.96 | 0.002 ⋆ |
| Platelet count | 0.004 | 1.00 | <0.001 ⋆⋆ |
| White Cell Count | 0.140 | 1.15 | 0.001 ⋆ |
| Neutrophil Count | 0.225 | 1.25 | <0.001 ⋆⋆ |
| Albumin | -0.131 | 0.88 | <0.001 ⋆⋆ |
| ALT | -0.030 | 0.97 | <0.001 ⋆⋆ |
| CRP | 0.022 | 1.02 | 0.001 ⋆ |
| ESR | 0.005 | 1.01 | 0.390 |
| Calprotectin | 0.000 | 1.00 | 0.854 |
| Ferritin | -0.003 | 1.00 | 0.005 ⋆ |
| Serum Iron | -0.072 | 0.93 | <0.001 ⋆⋆ |
| Transferrin Saturation | -0.026 | 0.97 | 0.017 ⋆ |
| Vitamin D | 0.008 | 1.01 | 0.277 |
| Serum Folate | -0.065 | 0.94 | 0.140 |
| Red Cell Folate | -0.001 | 1.00 | 0.100 |
| B12 | 0.001 | 1.00 | 0.043 ⋆ |

**Table 5.4**
Univariate Cox regression associating lvAUC values and OPO events.
† p-value for Log Rank test of association.
⋆ $p<0.05$
⋆⋆ $p<0.001$

| | $\beta$ coefficient | Hazard Ratio | p-value † |
|---|---|---|---|
| Age at Diagnosis | -0.012 | 0.99 | 0.054 |
| Sex (Female) | -0.004 | 1.00 | 0.979 |
| Date of Diagnosis | 0.000 | 1.00 | 0.633 |
| Perianal Disease at Diagnosis | -0.037 | 0.96 | 0.893 |
| Smoking at Diagnosis | 0.095 | 1.10 | 0.234 |
| Smoking at OPO event | 0.548 | 1.73 | 0.001 ⋆ |
| Previous OPO | 0.494 | 1.64 | 0.004 ⋆ |
| IV Steroids at Diagnosis | -0.234 | 0.79 | 0.273 |
| IL23R status (GG vs AG) | -0.152 | 0.86 | 0.701 |
| ATG16L1 status (Reference Level A) | | | |
| AG | 0.368 | 1.44 | 0.193 |
| G | 0.463 | 1.59 | 0.111 |
| Number of NOD2 Mutations (Reference Level 0) | | | |
| 1 | -0.170 | 0.84 | 0.397 |
| 2 | 0.037 | 1.04 | 0.899 |
| Montreal Location at Diagnosis (Reference Level L2) | | | |
| L1 | 0.637 | 1.89 | 0.004 ⋆ |
| L3 | 0.424 | 1.53 | 0.070 |

**Table 5.5**
Univariate cox regression: Other confounding variables.
† p-value for Log Rank test of association.
⋆ $p<0.05$

| | |
|---|---|
| Haemoglobin (Sex Adjusted) | $<129\ \mathrm{g\,L^{-1}}$ (m) |
| | $<114\ \mathrm{g\,L^{-1}}$ (f) |
| Haemoglobin | $<115\ \mathrm{g\,L^{-1}}$ |
| MCV | <86 fL |
| Platelet Count | $>370\ \mathrm{x10^9/L}$ |
| White Cell Count | $>11.5\ \mathrm{x10^9/L}$ |
| Neutrophil Count | $>8.6\ \mathrm{x10^9/L}$ |
| Albumin | $<37\ \mathrm{g\,L^{-1}}$ |
| ALT | <18 U/L |
| CRP | $>21\ \mathrm{mg\,L^{-1}}$ |
| ESR | >15 mm/h |
| Faecal Calprotectin | $>174\ \mathrm{\mu g\,g^{-1}}$ |
| Ferritin | $<53\ \mathrm{\mu g\,L^{-1}}$ |
| Iron | $<7\ \mathrm{\mu mol\,L^{-1}}$ |
| Transferrin Saturation | <13 % |
| Vitamin D | $<77\ \mathrm{nmol\,L^{-1}}$ |
| Serum Folate | $<15\ \mathrm{nmol\,L^{-1}}$ |
| Red Cell Folate | $<360\ \mathrm{nmol\,L^{-1}}$ |
| Vitamin B12 | $<335\ \mathrm{pmol\,L^{-1}}$ |

**Table 5.6**
Optimal cutoff for conversion from continuous to categorical lvAUC variables.

| | $\beta$ Coefficient | Odds Ratio | p-value † |
|---|---|---|---|
| lvAUC albumin level < 37 | 0.91 | 4.41 | <0.001 ⋆⋆ |
| lvAUC platelet count > 370 | 0.46 | 2.22 | 0.027 ⋆ |
| lvAUC MCV < 86 | 0.48 | 2.71 | 0.007 ⋆ |
| lvAUC neutrophil count > 8.6 | 1.87 | 5.39 | <0.001 ⋆⋆ |
| Montreal Location at Diagnosis (reference level = L2) | | | |
| L1 | 0.58 | 2.53 | 0.011 ⋆ |
| L3 | 0.30 | 1.24 | 0.217 |
| Previous Event | 0.48 | 2.87 | 0.004 ⋆ |

**Table 5.7**
Cox regression: multivariate analysis.
† Log Rank test.
⋆ p<0.05
⋆⋆ p<0.001
Included OPO events or censored observation periods =412.

| | No OPO | OPO | Sens | Spec | PPV | NPV | ⋆ Duration Observation |
|---|---|---|---|---|---|---|---|
| † 0 | 70 | 26 | 1.00 | 0.30 | 0.44 | 0.73 | 7.1 |
| 1 | 105 | 67 | 0.86 | 0.30 | 0.49 | 0.73 | 5.4 |
| 2 | 44 | 52 | 0.49 | 0.76 | 0.61 | 0.65 | 3.7 |
| 3 | 10 | 26 | 0.20 | 0.95 | 0.75 | 0.60 | 2.7 |
| 4 | 1 | 10 | 0.06 | 0.99 | 0.83 | 0.57 | 2.5 |
| 5 | 1 | 0 | 0.00 | 1.00 | 0.00 | 0.56 | 3.1 |

**Table 5.8**
Contingency table.
† Number of predictors (lvAUC albumin level, lvAUC platelet count, lvAUC MCV, lvAUC neutrophil count, ileal disease location, preveious event)
⋆ Median value in years.
n=418.
Sens=Sensitivity, Spec=Specificity, PPV=positive predictive value, NPV = negative predictive value.

## 5.8. Discussion

### 5.8.1. Prediction of OPO

In this study an lvAUC albumin $< 37\text{g}\,\text{L}^{-1}$, lvAUC platelet count $> 370$ x$10^9$/L, lvAUC MCV $< 86$ fL and an lvAUC neutrophil count $> 8.6$ x$10^9$/L were associated with subsequent development of an OPO, predominantly stenosis, in patients with Crohn's disease. These observations provide data to support the concept of using longitudinal laboratory testing to predict subsequent disease course in patients with Crohn's disease.

In our experience laboratory testing for patients with Crohn's disease tends to be reactive, performed to correlate current symptoms with objective inflammatory or nutritional markers, or to assess the response of these markers to a change in therapy. This study demonstrates that these same laboratory variables can provide an objective longitudinal assessment of inflammatory and nutritional status, an assessment which correlates with the subsequent development of an OPO. It is noteworthy that the cut-off lvAUC values that were associated with OPO for each variable in our study were not markedly abnormal when compared to normal ranges for individual results. Figure 5.5 demonstrates that lvAUC is a markedly different entity to a single laboratory value, and that only with consistent and significant abnormality in individual results is an lvAUC value moved away from normality. lvAUC data cannot be compared to single laboratory results, and must be considered without referring to the normal range for individual results.

Faecal calprotectin was used routinely in the management of our patients from 2008, and therefore a small number of observation periods had faecal calprotectin data to use for analysis. There were not enough data, and not enough statistical power, to adequately assess this biomarker which may provide prognostic information in future studies.

The aim of this analysis was to identify persistent laboratory abnormalities which were associated with a clinically significant outcome event. The dominant observed OPO was stenosis, partly due to the exclusion of half of all observed fistulizing or penetrating complications because they occurred within 9 months of diagnosis. The absolute rate and relative proportion of intra-abdominal stenosis, perforation and fistula formation in our cohort is similar to published cohorts when represented by cumulative Montreal phenotype classification.(Silverberg et al. 2005; Tarrant et al. 2008) We believe that this is representative of the spectrum of long term complications suffered by patients with Crohn's disease, and that therapy initiated with the goal of preventing the development of long term complications would be given predominantly to prevent bowel stenosis.

### 5.8.2. Objectivity In outcome assessment

Intestinal surgery is a commonly used endpoint in longitudinal studies of Crohn's disease.(Ramadas et al. 2010; Chatu et al. 2014; Feagan, Panaccione, et al. 2008) We elected to use development of OPO and not performance of intestinal surgery as our primary endpoint in this study as we consider that the decision to perform surgery may be influenced by subjective factors (physician and surgeon opinion of what is a reasonable indication for surgery, patient agreement to undergo surgery) and may not be a truly objective marker of outcome. This is demonstrated by varied rates of intestinal surgery in cohorts of patients with Crohn's disease, across time periods and across geographic location.(Wolters, M. G. V. M. Russel, and Stockbrügger 2004; Ramadas et al. 2010) The international IBD community have proposed a more objective definition of long term outcome in Crohn's disease, the Lemann score.(Pariente, Mary, et al. 2015) This score assigns a numeric representation of outcome based on observation of stenosis, perforation and fistulization made at ileocolonoscopy, gastroscopy, surgery, on examination of macroscopic histological specimens, clinical examination and cross sectional imaging. It is complex and has not yet been routinely taken up in observational Crohn's disease research to date. However, this score is likely to provide a more objective and standardized

measure of longitudinal outcome in Crohn's disease, which may translate better between patients in different time periods and different geographic locations, and allow closer comparison between different research cohorts.

### 5.8.3. Weaknesses in study design

This was an observational study and so was inherently subject to bias. We have attempted to minimize bias through study design, although acknowledge that unidentified bias may have influenced the observed associations.

Delay in diagnosis of perforation, fistula or stenosis mean that the observed association between abnormal tests and OPO may be due to an undiagnosed OPO being present when the tests were performed. This would reduce this study to observing that patients with an OPO have abnormal laboratory values. We attempted to minimize this bias by excluding laboratory results taken in the 90 days leading up to the first identification of an OPO. We also excluded results taken within 90 days of diagnosis, where we feel actual or imminent OPO is common and often not observed on initial testing.

Laboratory tests performed near the time of abdominal surgery were excluded from the analysis. Surgery could be considered a surrogate marker for a more severe phenotype, and this exclusion reduced the possible bias introduced by surgery itself causing abnormal lab tests. Without this restriction a true association between intestinal surgery and subsequent development of an OPO could be interpreted as an association between abnormal blood results (consequent to surgery) and the subsequent development of an OPO.

When this analysis was run including all laboratory results the associations were similar. However, because of the biases mentioned above, we feel that the exclusions used mean it is more likely we have observed a true association between laboratory testing taken when a patient is free of an OPO, and the subsequent development of an OPO.

Many of our patients suffered OPO at diagnosis or early in their disease course and then were observed for many years. We used the described study design to allow inclusion of data collected subsequent to a resolved OPO, correlating it with the subsequent development of a new OPO. Because of this study design, single patients may have overly influenced the observed result by contributing multiple OPO events to the analysis - 48 patients contributed more than one OPO event to the analysis. This influence was controlled for by including a relatedness variable (experience of previous OPO) in the final multivariate model. Without this study design the application of this data would be limited to patients early in their disease course. The described study design allows translation of the observed associations to patients both early and late in their disease course, and to patients who have suffered a previous OPO.

Laboratory tests were more likely to be performed frequently when patients were unwell. Patients taking immunomodulatory therapy also had blood tests regularly taken when they were well, to monitor for medication side effects. As a potential source of bias this was minimized by using all available laboratory data which met temporal criteria, including those requested by general practitioners. Secondly, we feel that using lvAUC to represent laboratory variables gave a longitudinal assessment of inflammatory and nutritional status, less influenced by clusters of multiple testing at times when patients were unwell.

MCV is influenced by thiopurine and methotrexate immunotherapy. It is possible that an association between a low MCV and subsequent OPO occurrence is due to the effect of these drugs on both the MCV, and the natural history of the disease. This criticism does not extend to albumin level, platelet count or neutrophil count.

Patient reported symptoms of diarrhoea and pain were not collected in this study, and we therefore do not know if the observed associations are independent of patient symptoms. For patients with Crohn's disease with significant symptoms attributable to their disease, estimation of future complication risk contributes little to selection of treatment. These patients are likely to want to take stronger

therapy to control their symptoms. It is for patients without marked symptoms that estimation of future complication risk, such as that provided by longitudinal laboratory data, would be most useful. We felt that collection of patient reported symptoms through retrospective clinical review would produce unreliable data, and we elected not to do it. This meant that all analyzed factors were objective, however we do not know if they were independent of patient symptoms.

## 5.9. Conclusion

A consistently low serum albumin level, consistently high platelet count, consistently low MCV and consistently high neutrophil count were associated with subsequent OPO, predominantly stenosis, in patients with Crohn's disease. In addition to recognized markers of poor outcome in Crohn's disease, these laboratory tests may prove to be useful as markers of risk of development of subsequent OPO, and provide a rationale for escalation of therapy. These findings require validation in an external cohort.

# 6. A consistently low serum albumin or high C reactive protein is associated with subsequent development of perianal fistulae in patients with Crohn's disease.

## 6.1. Authors

James Irwin, (1,2,3) Emma Ferguson, (2) Lisa A Simms, (3) Katherine Hanigan, (3) Graham Radford-Smith (1,2, 3)

## 6.2. Institutions

1. Department of Gastroenterology and Hepatology, Royal Brisbane and Women's Hospital, Brisbane, Australia.

2. School of Medicine, The University of Queensland, Brisbane, Australia.

3. Inflammatory Bowel Diseases Research Group, QIMR Berghofer Medical Research Institute, Brisbane, Australia

## 6.3. Abstract

*Aim:* To study the correlation between longitudinal laboratory testing and subsequent development of perianal fistulae in patients with Crohn's disease.

*Methods:* Patients diagnosed with Crohn's disease between 1st Jan 1994 and 1st March 2008, with more than five years of clinical follow-up, were analyzed. Patients developing a perianal fistula within 9 months of diagnosis were excluded. Laboratory data were represented by the area under the curve of values measured in the complication free period leading up to development of a perianal fistula. Association between laboratory values (C reactive protein, erythrocyte sedimentation rate, platelet count, haemoglobin level, mean cell volume, white blood cell count, neutrophil count, albumin, alanine transaminase, vitamin D, vitamin B12, folate, faecal calprotectin, serum iron, transferrin saturation and ferritin level) and perianal fistula development was analyzed using Cox regression.

*Results:* 268 patients met inclusion criteria and were observed for a median of 9.35 (Interquartile range (IQR) 6.41-13.50) years. 49 developed a perianal fistula a median 4.43 years (IQR 3.10-8.54 years) after diagnosis. Blood testing was performed a median of 4.33 (IQR 2.88-6.66) times per year for each patient. After multivariate analysis with inclusion of potentially confounding variables, a CRP consistently >11 mg/L (Hazard ratio (HR) 2.63, p=0.003), an albumin level consistently <38 g/L (HR 2.66, p=0.002), age at diagnosis <32 (HR 3.86, p=0.005) and L2 Montreal disease location (HR 2.39, p=0.017) were independently associated with development of perianal fistulae.

*Conclusion:* An albumin consistently <38 g/L or a CRP consistently >11 mg/L are associated with subsequent development of perianal fistulae in patients with Crohn's disease. Serial monitoring and longitudinal analysis of these variables may aid in identifying patients at risk of developing perianal fistulae, and allow better tailoring of preventative therapy. These findings need validation in an external cohort.

## 6.4. Introduction

Crohn's disease is a chronic inflammatory condition of the human gastrointestinal tract of undetermined aetiology. It is characterized by a relapsing and remitting course (Munkholm et al. 1995) and by significant morbidity from chronic abdominal pain, diarrhoea, perianal abscess and fistula formation, bowel stenosis and obstruction, internal fistulization and bowel perforation.(Lazarev et al. 2010; Cosnes, Nion-Larmurier, et al. 2005; Ramadas et al. 2010; Tarrant et al. 2008; Thia et al. 2010; Magro et al. 2014) Chronic perianal fistulae may be present for many years in patients with Crohn's disease, and cause significant morbidity from pain, faecal leakage and anal stenosis. Perianal fistulae are present in up to 20% of patients at diagnosis, and develop in a further 10% over the next five years.(Schwartz et al. 2002; Cosnes, S. Cattan, et al. 2002)

Infliximab and adalimumab have been shown to aid healing of perianal fistulae.(Present et al. 1999; J.-F. Colombel et al. 2007) In a meta analysis, thiopurine use was associated with resolution of perianal fistulae, although this effect has not been demonstrated as a primary endpoint in a controlled trial.(Pearson, May, G. H. Fick, et al. 1995) The current paradigm of medical management in Crohn's disease is that enduring control of bowel inflammation reduces tissue damage, and reduces long term risk of complications such as bowel perforation or stenosis. There are no published data demonstrating that immunomodulatory therapy reduces the risk of developing a perianal fistula. However, it is attractive to consider that such complications may be prevented with medical therapy.

Immunomodulatory therapy in Crohn's disease carries a significant side effect profile. For patients without significant symptoms from their Crohn's disease, a decision to embark on long term immunomodulatory therapy rests on assessment of risk of development of a poor long term outcome, and the magnitude of expected reduction in this risk associated with medication use. Risk of development of perianal fistulae forms part of this assessment.

Most published data observe that perianal fistulae are more commonly observed in patients with colonic disease location.(D. R. Williams et al. 1981; Rankin et al. 1979; Veloso et al. 2001; Tang, Rawsthorne, and Bernstein 2006) Data demonstrating either no association between disease location and development of perianal fistulae,(Lapidus 2006) or an association between ileal disease location and perianal disease (Eglinton et al. 2012) have also been published. In both of these studies a markedly high proportion of each cohort had L2 Montreal location of disease (52% and 42% respectively). Perianal location of disease forms part of the diagnostic criteria for Crohn's disease.(Lennard-Jones 1989) Intestinal penetrating or stenotic complications, and associated surgery are less common in colonic Crohn's disease. Hence a transmural intestinal specimen is less commonly available to aid diagnosis in colonic inflammatory bowel disease, and diagnosis depends more heavily on assessment of disease location and mucosal histology. These observations suggest that the association between location of disease and perianal fistula formation is dependent on how colonic inflammatory bowel disease is classified in a cohort. Stringent interpretation of a diagnosis of Crohn's disease will require the presence of extracolonic disease (perianal, small bowel or upper gastrointestinal) or granulomas on biopsy in addition to colonic disease location and histological evidence of chronic inflammation. Less stringent interpretation may require varying degrees of discontinuous colonic involvement and histological evidence of chronic inflammation only. Differences in interpretation of diagnostic criteria for colonic Crohn's disease are likely to explain differing association of disease location with perianal disease between cohorts.

par

Young age at diagnosis and complicated intestinal disease (perforation, fistula formation or stenosis) have also been associated with perianal fistula formation.(Eglinton et al. 2012; Lapidus 2006)

Published literature associating laboratory testing and long term outcome in Crohn's disease demonstrate an association between a high CRP at diagnosis, and a high platelet count at diagnosis, with subsequent intestinal surgery.(Loly, Belaiche, and Louis 2008; Henriksen et al. 2008) We are not aware of published

data analyzing the association between laboratory testing and subsequent perianal fistula formation. Data allowing further stratification of patients into those more and less likely to develop a perianal fistula could allow improved tailoring of immunomodulatory therapy in Crohn's disease. Longitudinal laboratory testing is a routine part of the management of patients with Crohn's disease. Testing is performed to assess inflammatory status, nutritional status, and to monitor for side effects from prescribed medications. These results represent a silo of objective clinical information which may provide useful prediction of future disease course. We hypothesize that these results contain a measurable signal that is associated with active and progressive Crohn's disease, and that this signal is associated with the subsequent development of a perianal fistula.

## 6.5. Materials and methods

### 6.5.1. Patients

This was a single centre observational longitudinal cohort study. All patients with a diagnosis of Crohn's disease made between 1$^{st}$ January 1994 and 31$^{st}$ March 2008, managed at the Inflammatory Bowel Diseases Unit at the Royal Brisbane and Women's Hospital (RBWH, Brisbane, Australia), were invited to participate in the research programme. Serological, epidemiological, clinical and genetic data were obtained for each patient. Further clinical data was obtained through retrospective review of the clinical record.

### 6.5.2. Inclusion and exclusion criteria

All patients met criteria for a diagnosis of Crohn's disease. (Lennard-Jones 1989) These were essentially the Lennard-Jones criteria, however patients who did not undergo surgery, and therefore did not have a surgical specimen for examination, were not required to demonstrate evidence of transmural bowel involvement. For

these patients evidence of chronic mucosal bowel inflammation in a typical distribution (ileal only, or non-continuous colonic) was considered adequate for diagnosis. All early clinical information was reviewed to confirm that diagnostic criteria were met. Patients who did not have 5 years of follow-up data available were not included in the analysis. Patients who developed a perianal fistula within 270 days of diagnosis were excluded from analysis. This exclusion was made because little longitudinal laboratory data were available for these patients.

### 6.5.3. Definitions

The date of diagnosis was defined as the first date when the patient was felt to meet diagnostic criteria for Crohn's disease by the treating physician.(Lennard-Jones 1989) This was usually when tissue was first obtained at either colonoscopy or surgery, confirming chronic bowel inflammation in the setting of chronic abdominal symptoms. A perianal fistula was defined as a sinus in the perianal skin weeping faeces, pus or serous fluid. A perianal abscess without an identified sinus tract was not considered a perianal fistula. Perianal fistulae could be identified on clinical examination, at perianal surgery, on pelvic magnetic resonance imagining (MRI) or endoanal ultrasound. The observation period prior to the development of a perianal fistula in which laboratory data were examined was defined as follows; 90 days following date of diagnosis, ninety days prior to first identification of a perianal fistula and ninety days prior to censoring at the end of the study period if no perianal fistula developed over the observation period. Laboratory variables taken within ninety days of abdominal surgery were excluded.

### 6.5.4. Laboratory data

Laboratory data were obtained by matching identities to four external laboratory databases: AUSLAB (all laboratory results performed in public hospitals across Queensland 1$^{st}$ Jan 1999 to present), PARIS (a historical database which records all laboratory results performed in public hospitals in Brisbane 1$^{st}$ Jan 1985 - 1$^{st}$

$$lvAUC = \sum_{1}^{n-1} (time_{x+1} - time_x) \times \left( \min(value_x, value_{x+1}) + \frac{|value_{x+1} - value_x|}{5} \right)$$

**Figure 6.1**
Area under curve calculation: for variables that usually rise with inflammation or malnutrition (platelet count, CRP, ESR, WBC count, neutrophil count, faecal calprotectin, ALT)
min = minimum
max = maximum

Jan 1999), Queensland Medical Laboratories (QML, private laboratory database covering all of Queensland 1st Jan 1995 to present) Sullivan and Nicholaides Pathology (SNP, private laboratory database covering all of Queensland 1st Jan 2001 - present). QML and SNP are the two major private pathology providers in Queensland. Patients were matched by surname, first name, date of birth (DOB) and sex.

C reactive protein (CRP, mg $L^{-1}$), erythrocyte sedimentation rate (ESR, mm/h), haemoglobin level (g $L^{-1}$), white blood cell count (WBC, x$10^9$/L), platelet count (x$10^9$/L), neutrophil count (x$10^9$/L), mean cell volume (MCV, fL), faecal calprotectin (µg $g^{-1}$ faeces), albumin (g $L^{-1}$), alanine transaminase (ALT, U/L), serum ferritin (mg $L^{-1}$), serum iron (µmol $L^{-1}$), transferrin saturation (%), vitamin D level (nmol $L^{-1}$), red cell folate (nmol $L^{-1}$), serum folate (nmol $L^{-1}$) and vitamin B12 level (pmol $L^{-1}$) were analyzed. When analyzed as continuous variables, values reported as below the lowest detectable level were considered equal to zero, while all values reported above a highest detectable level were considered equal to that highest detectable level. These considerations were most important for CRP which had a lower limit of detection of 5mg/L in the 1990's, reducing to 2mg/L in 2006.

A representative value (laboratory variable Area Under Curve, lvAUC) for each laboratory variable, for each observation period, was calculated using a variation of the Area Under the Curve (AUC) using all eligible dated laboratory values ordered temporally ($value_n$ taken at $time_n$, see figures 6.1, 6.2).

$$lvAUC = \sum_{1}^{n-1} (time_{x+1} - time_x) \times \left( \max(value_x, value_{x+1}) - \frac{|value_{x+1} - value_x|}{5} \right)$$

**Figure 6.2**
Area under curve calculation: for variables that usually fall with inflammation or malnutrition (albumin, haemoglobin, ferritin, serum iron, MCV, transferrin saturation, serum folate, red cell folate, vitamin D, vitamin B12)

### 6.5.5. Statistical analysis

Statistical analysis was performed in the R statistical computing environment. (R Core Team 2014) Correlation between lvAUC values and first perianal fistula was analyzed using Cox regression. In the exploratory analysis, the lvAUC for each variable observed over the immediately preceding observation period was correlated with perianal fistula formation using univariate Cox regression. lvAUC variables with a p-value of association $<0.2$ were then entered into a multivariate model in a stepwise fashion, retaining those with independent correlation ($p<0.05$). To make the analysis more clinically relevant, laboratory variables were converted to dichotomous categorical variables, with optimized cut-off values determined using an automated algorithm.(see coding reference 15) These relationships were then adjusted for potential confounding by including the following variables in a final multivariate model: age at diagnosis, sex, date of diagnosis, Montreal classification disease location at diagnosis,(Silverberg et al. 2005) smoking status at diagnosis, ATG16L1 genotype, NOD2 genotype, IL23R genotype, and use of intravenous steroids at first disease flare.

### 6.5.6. Ethical considerations

Ethical approval for the study was obtained through the RBWH ethics committee. All participating patients consented to take part in the unit's research programme. Consent was given for the use of routine clinical and laboratory data for research purposes. It also covered the collection of serum, whole blood and bowel tissue samples for research purposes.

## 6.6. Results

Of 360 patients assessed, 302 were observed for 5 or more years. 28 patients developed their first perianal fistula within 270 days of diagnosis and were excluded. 6 patients developed a perianal fistula with no preceding laboratory data and were excluded. 268 patients were included in the analysis of whom 49 developed a perianal fistula a median 4.43 years (Intraquartile range IQR 3.10-8.54 years) after diagnosis.(see Figure 6.3) The demographics of these patients are shown in Table 6.1. The median number of laboratory results for each test during each observation period is tabulated in Table 6.2. Data for faecal calprotectin were available for 1/3 of all observation periods.

lvAUC values for all analyzed laboratory variables are shown in Table 6.3, stratified by whether the observation period ended in a perianal fistula or in censoring. Figure 6.4 demonstrates the relationship between raw CRP results and the lvAUC value over one observation period for one patient. Correlation using Cox regression between continuous lvAUC laboratory values and development of perianal fistulae are tabulated in Table 6.4. Correlation between possible confounding variables and perianal fistula are tabulated in Table 6.5. L2 Montreal location at diagnosis was positively correlated, while there was a trend for younger age and female sex to be correlated with development of perianal fistulae. Optimal cutoff values for conversion of continuous to categorical laboratory variables are shown in Table

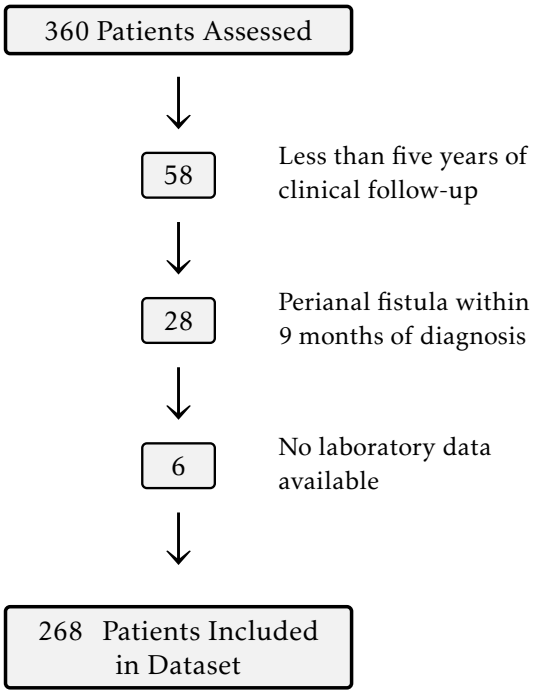


**Figure 6.3**
Exclusions.

6.6. A final multivariate model of independent variables is tabulated in Table 6.7. Table 6.8 shows the sensitivity, specificity, positive and negative predictive value of these features in prediction of development of perianal fistula when applied to the derivation cohort.

| Demographics at Diagnosis | |
|---|---|
| Age (mean) | 27.1 |
| Female | 57.09 % |
| Caucasian Australian | 85.45 % |
| Smoker | 51.87 % |
| Family History IBD | 27.99 % |
| Follow-up (median, years) | 10.64 |
| | |
| Montreal location (at diagnosis) | |
| L1 | 120 |
| L2 | 47 |
| L3 | 98 |
| No macroscopic ileocolonic disease | 3 |

**Table 6.1**
Demographics.

| | Platelet Count | Haemoglobin | Albumin Level | CRP | ESR | Faecal Calprotectin |
|---|---|---|---|---|---|---|
| Proportion of patients with results | 1 | 1 | 1 | 0.99 | 0.98 | 0.39 |
| Median number of results per observation period | 30 (16-48) | 30 (16-48) | 26 (13-42) | 20 (10-34) | 17 (8-26) | 4 (2-6) |
| Median tests per year | 4.33 (2.88-6.66) | 4.35 (2.88-6.65) | 3.89 (2.54-6.18) | 3.47 (2.18-5.38) | 2.99 (2.04-4.72) | 1.81 (1.25-2.66) |

**Table 6.2**
Number of laboratory tests (prior to development of fistula, or censuring at study end), used to calculate lvAUC value for each patient. Number of perianal fistula events = 49.

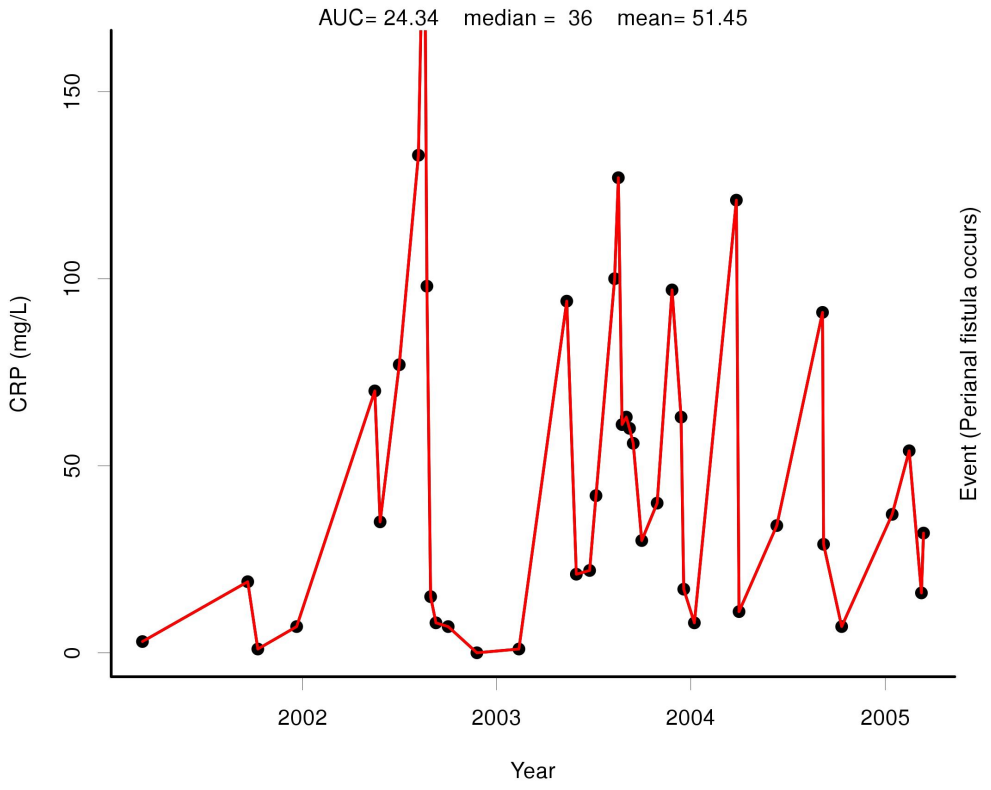


**Figure 6.4**
Derivation of the lvAUC value for a single sequence of CRP results.

| Variable | Perianal Fistula | No Perianal Fistula | p-value (t-test) |
|---|---|---|---|
| Haemogobin ($g\,L^{-1}$) | 130.6 (122.07 - 137.95) | 134.92 (127.24 - 143.9) | 0.027* |
| MCV (fL) | 88.9 (84.65 - 92.17) | 89.99 (87.02 - 93.43) | 0.06 |
| Platelet count ($x10^9/L$) | 313.55 (285.58 - 339.46) | 281.27 (240.89 - 324.71) | 0.006* |
| White Cell Count ($x10^9/L$) | 7.88 (6.21 - 9.23) | 7.26 (6.08 - 8.39) | 0.094 |
| Neutrophil Count ($x10^9/L$) | 5.36 (4.26 - 6.51) | 4.72 (3.77 - 5.67) | 0.014* |
| Albumin ($g\,L^{-1}$) | 39.81 (37.42 - 41.33) | 41.27 (39.61 - 43.16) | 0.002* |
| ALT (U/L) | 15.24 (10.72 - 19.61) | 17.65 (13.71 - 23.5) | 0.006* |
| CRP ($mg\,L^{-1}$) | 12.62 (6.2 - 20.03) | 6.04 (2.65 - 11.01) | 0.001* |
| ESR (mm/h) | 21.9 (13.73 - 33.32) | 12.41 (7.72 - 20.58) | 0.001* |
| Calprotectin ($\mu g\,g^{-1}$) | 292.18 (212 - 780) | 138.28 (59.86 - 333.33) | 0.252 |
| Ferritin ($\mu g\,L^{-1}$) | 59.97 (35.26 - 135.48) | 71.52 (44.39 - 109.83) | 0.547 |
| Serum Iron ($\mu mol\,L^{-1}$) | 9.68 (6.52 - 13.05) | 13.56 (10.39 - 17.11) | <0.001 ** |
| Transferrin Saturation (%) | 17.03 (12.36 - 24.21) | 21.15 (15.74 - 27.46) | 0.002* |
| Vitamin D ($nmol\,L^{-1}$) | 68.6 (56.6 - 94) | 81.22 (67.12 - 96.03) | 0.642 |
| Serum Folate ($nmol\,L^{-1}$) | 34.78 (34.26 - 35.3) | 33.52 (28.49 - 41.96) | 0.716 |
| Red Cell Folate ($nmol\,L^{-1}$) | 934.32 (756.72 - 1434.95) | 1092.32 (892.65 - 1389) | 0.918 |
| B12 ($pmol\,L^{-1}$) | 343.32 (299.05 - 575.35) | 345.82 (253.78 - 452.09) | 0.108 |

**Table 6.3**
Comparison of lvAUC values over observation periods prior to perianal fistula development, with lvAUC values over observation periods prior to censoring.
lvAUC values reported as Median (Lower Quartile - Upper Quartile)
p-value is for a two tailed t-test comparing the difference of two means.

| | $\beta$ coefficient | Hazard Ratio | p-value † |
|---|---|---|---|
| Serum Iron | -0.167 | 0.85 | <0.001 ⋆⋆ |
| Albumin | -0.141 | 0.87 | 0.001 ⋆ |
| Transferrin Saturation | -0.067 | 0.94 | 0.007 ⋆ |
| MCV | -0.051 | 0.95 | 0.041 ⋆ |
| ALT | -0.030 | 0.97 | 0.039 ⋆ |
| Haemogobin | -0.029 | 0.97 | 0.020 ⋆ |
| Vitamin D | -0.011 | 0.99 | 0.475 |
| Ferritin | -0.002 | 1.00 | 0.407 |
| Red Cell Folate | 0.000 | 1.00 | 0.854 |
| B12 | 0.001 | 1.00 | 0.091 |
| Calprotectin | 0.002 | 1.00 | 0.057 |
| Platelet count | 0.004 | 1.00 | 0.027 ⋆ |
| Serum Folate | 0.018 | 1.02 | 0.771 |
| ESR | 0.033 | 1.03 | 0.001 ⋆ |
| CRP | 0.060 | 1.06 | <0.001 ⋆⋆ |
| White Cell Count | 0.154 | 1.17 | 0.051 |
| Neutrophil Count | 0.288 | 1.33 | 0.003 ⋆ |

**Table 6.4**
Univariate Cox regression associating continuous lvAUC values and perianal fistula events.
† p-value for Log Rank test of association.
⋆ p<0.05
⋆⋆ p<0.001

| | $\beta$ coefficient | Hazard Ratio | p-value † |
|---|---|---|---|
| Age at Diagnosis | 0.000 | 1.00 | 0.072 |
| Sex (Male) | -0.022 | 0.98 | 0.085 |
| Date of Diagnosis | -0.253 | 0.78 | 0.108 |
| Perianal Disease at Diagnosis | -0.416 | 0.66 | 0.164 |
| Smoking at Diagnosis | -0.826 | 0.44 | 0.222 |
| Smoking at OPO event | 0.245 | 1.28 | 0.448 |
| IV Steroids at Diagnosis | 0.131 | 1.14 | 0.707 |
| IL23R status (GG vs AG) | 0.000 | 1.00 | 1.000 |
| ATG16L1 status (Reference Level A) | | | |
| AG | -0.161 | 0.85 | 0.710 |
| G | -0.825 | 0.44 | 0.102 |
| Number of NOD2 Mutations (Reference Level 0) | | | |
| 1 | 0.293 | 1.34 | 0.407 |
| 2 | -0.521 | 0.59 | 0.479 |
| Montreal Location at Diagnosis (Reference Level L1) | | | |
| L2 | 1.100 | 3.00 | 0.002 $\star$ |
| L3 | 0.488 | 1.63 | 0.163 |

**Table 6.5**
Univariate cox regression: Other confounding variables.
† p-value for Log Rank test of association.
$\star$ $p<0.05$

| | |
|---|---|
| Haemoglobin (Sex Adjusted) | $<134\ \mathrm{g\,L^{-1}}$ (m) |
| | $<119\ \mathrm{g\,L^{-1}}$ (f) |
| Haemoglobin | $<135\ \mathrm{g\,L^{-1}}$ |
| MCV | <86 fL |
| Platelet Count | $>270\ \mathrm{x10^9/L}$ |
| White Cell Count | $>8.4\ \mathrm{x10^9/L}$ |
| Neutrophil Count | $>5.9\ \mathrm{x10^9/L}$ |
| Albumin | $<38\ \mathrm{g\,L^{-1}}$ |
| ALT | <12 U/L |
| CRP | $>11\ \mathrm{mg\,L^{-1}}$ |
| ESR | >25 mm/h |
| Faecal Calprotectin | $>198\ \mathrm{\mu g\,g^{-1}}$ |
| Ferritin | $<32\ \mathrm{\mu g\,L^{-1}}$ |
| Iron | $<7\ \mathrm{\mu mol\,L^{-1}}$ |
| Transferrin Saturation | <11 % |
| Vitamin D | $<60\ \mathrm{nmol\,L^{-1}}$ |
| Serum Folate | $<32\ \mathrm{nmol\,L^{-1}}$ |
| Red Cell Folate | $<810\ \mathrm{nmol\,L^{-1}}$ |
| Vitamin B12 | $<285\ \mathrm{pmol\,L^{-1}}$ |

**Table 6.6**
Optimal cutoff for conversion from continuous to categorical lvAUC variables.

| | Odds Ratio | p-value † |
|---|---|---|
| Albumin < 38 | 2.66 | 0.002 ⋆ |
| CRP > 11 | 2.63 | 0.003 ⋆ |
| Age < 32 | 3.86 | 0.005 ⋆ |
| Montreal Location at Diagnosis | | |
| (reference level = L1) | | |
| L2 | 2.39 | 0.017 ⋆ |
| L3 | 0.99 | 0.979 |

**Table 6.7**
Cox regression: Multivariate analysis
† Log Rank test
⋆ p<0.05
⋆⋆ p<0.001
Included Patients =263; Fistula events =47

| | No Fistula | Fistula | Sensitivity | Specificity | PPV | NPV | ⋆ Duration Observation |
|---|---|---|---|---|---|---|---|
| † 0 | 38 | 0 | | | | | 7.6 |
| 1 | 111 | 13 | 1.00 | 0.18 | 0.21 | 1.00 | 7.5 |
| 2 | 52 | 18 | 0.72 | 0.69 | 0.34 | 0.92 | 7.3 |
| 3 | 14 | 11 | 0.34 | 0.93 | 0.52 | 0.87 | 6.1 |
| 4 | 1 | 5 | 0.11 | 1.00 | 0.83 | 0.84 | 2.1 |

**Table 6.8**
Contingency table.
† Number of predictors (CRP, albumin level, colonic disease location, age)
⋆ Median value in years.
n=263
PPV=positive predictive value; NPV = negative predictive value.

## 6.7. Discussion

In this study longitudinally measured serum albumin and CRP correlate with subsequent development of perianal fistula in patients with Crohn's disease. An albumin consistently less than 38 g/L and a CRP consistently higher than 11 mg/L were the cutoff levels most strongly correlated with perianal fistula development. In addition to young age at diagnosis, complicated disease phenotype and colonic location of disease,(Eglinton et al. 2012; Lapidus 2006) these variables may allow further prediction of risk of perianal fistula formation, and allow more accurate tailoring of preventative medical and/or surgical therapy.

In our experience laboratory testing for patients with Crohn's disease tends to be reactive, performed to correlate current symptoms with objective inflammatory or nutritional markers, or to assess the response of these markers to a change in therapy. This study demonstrates that these same laboratory variables can provide an objective longitudinal assessment of inflammatory and nutritional status, an assessment which correlates with the subsequent development of a perianal fistula. It is noteworthy that the cut-off lvAUC values that were associated with perianal fistula formation for these variables in our study were not markedly abnormal when compared to normal ranges for individual results. Figure 6.4 demonstrates that lvAUC is a markedly different entity to a single laboratory value, and that only with consistent and significant abnormality in individual results is an lvAUC value moved away from normality. lvAUC data cannot be compared to single laboratory results, and must be considered without referring to to the normal range for individual results.

Faecal calprotectin was used routinely in the management of our patients from 2008, and therefore a small number of observation periods had faecal calprotectin data to use for analysis. There were not enough data, and not enough statistical power, to adequately assess this biomarker, which may provide prognostic information in future studies.

### 6.7.1. Influence of diagnostic criteria

L2 location Crohn's disease often requires disease location outside the ileum and colon (perianal or upper GI involvement) to meet diagnostic criteria (Lennard-Jones 1989) and distinguish it from ulcerative colitis. L1 and L3 location Crohn's disease do not rely on the presence of disease outside the ileum and colon to meet diagnostic criteria. Because of this there is a selection bias associating L2 location with perianal disease. In this cohort all patients with perianal fistulae within 9 months of diagnosis were excluded, meaning all patients with L2 disease location met diagnostic criteria without the presence of perianal fistulae. Despite this exclusion, there was still an association between subsequent perianal fistula formation and colonic disease location. This temporal relationship between the diagnosis of Crohn's disease and subsequent perianal fistula formation provides some evidence that colonic disease location is truly associated with perianal fistula formation, and that this association is not the result of how diagnostic criteria are defined.

L2 disease extent, young age at diagnosis and complex intestinal disease have previously been published as predictors of development of perianal fistula formation or of perianal disease.(Eglinton et al. 2012; Rankin et al. 1979; Lapidus 2006; D. R. Williams et al. 1981; Veloso et al. 2001; Tang, Rawsthorne, and Bernstein 2006) A consistently low albumin level and high CRP level are abnormalities that would be expected to be associated with chronic inflammation. Patients who developed a perianal fistula in this study did not have significant perianal disease prior to its development. These data suggest that perianal fistula formation is more likely to occur in patients with chronic inflammation of the ileum or colon. This interpretation of the data is consistent with the observation made by Eglinton *et al.* that perianal disease is more common in patients with complex intestinal disease.(Eglinton et al. 2012)

### 6.7.2. Study weaknesses

This was an observational study and so was inherently subject to bias. We have attempted to minimize bias through study design, although acknowledge that unidentified bias may have influenced the observed associations.

42/91 of patients who developed a fistula were excluded from this analysis, either because they developed a fistula early in their disease course, or because no laboratory data were available prior to fistula development. This reduced the statistical power of the study, and may have missed observing factors associated with early fistula development. These problems are inherent in the study design, which was structured to meet the primary aim of assessing the relationship between longitudinal laboratory test results and subsequent perianal fistula formation.

It is possible there was delay between development of a perianal fistula and its subsequent diagnosis. If this occurred with some frequency then this study would be reduced to observing that low serum albumin and high serum CRP are associated with active perianal disease. This possibility has been reduced by excluding laboratory tests performed within the 3 months prior to fistula development. Laboratory tests performed within 3 months of diagnosis were also excluded, considering that there are a high number of complications in the months following diagnosis, many of which are not immediately identified.

It is possible that the performance of abdominal surgery could confound the relationship between laboratory results and subsequent perianal fistula formation. Laboratory results taken within 3 months of abdominal surgery were excluded to minimize this possibility.

## 6.8. Conclusion

An albumin level consistently below 38 g/L or a CRP consistently above 11 mg/L are associated with subsequent perianal fistula formation in patients with Crohn's disease. These laboratory results may provide additional prognostication of clinical course for patients with Crohn's disease, and allow better tailoring of preventative therapy. These results require validation in an external cohort.

# 7. Further Longitudinal Analyses

We have accumulated a detailed longitudinal database on the RBWH cohort of patients with Crohn's disease. I have become very familiar with these data and with methods of structuring and analyzing them in the statistical programming environment R. This has led to a number of observations which we hope will yield fruitful insight into the natural history of Crohn's disease. Three of these analyses are briefly outlined here.

The common thread which links these analyses to this thesis is that they all interpret clinical questions through longitudinal analysis of objective data.

## 7.1. Longitudinal disease progression

Cosnes et al. published a paper describing the natural history of Crohn's disease in 2002 in Inflammatory Bowel Diseases, using the Vienna classification system. (Cosnes, S. Cattan, et al. 2002; Gasche et al. 2000) This paper and others that followed introduced the concept that Crohn's disease is a chronic and progressive disease, with patients moving from uncomplicated inflammatory disease to more complicated disease (stricturing or penetrating) over time.

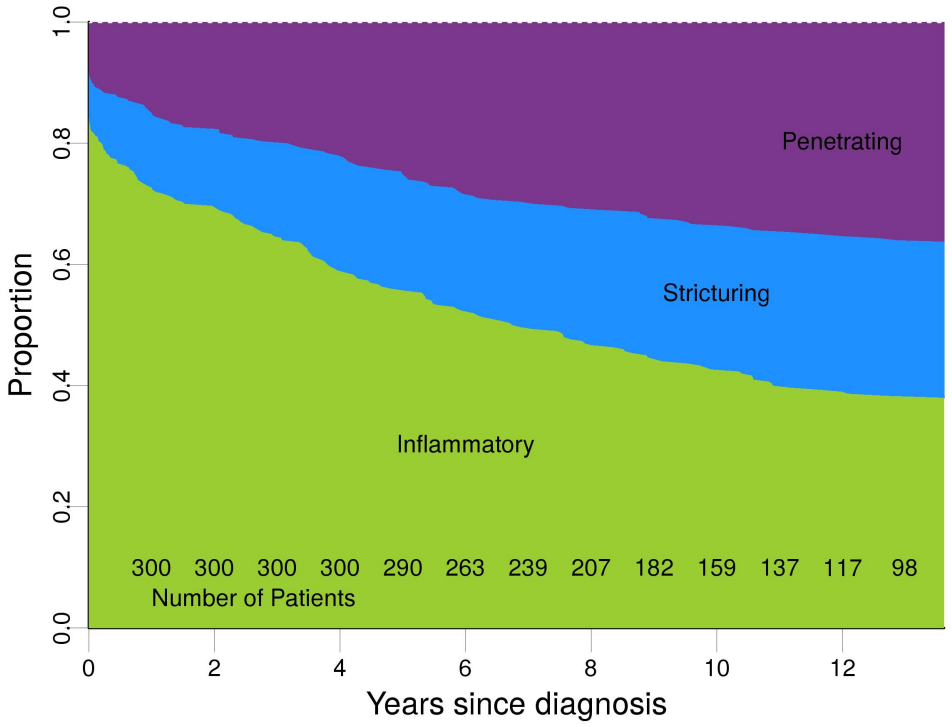


**Figure 7.1**
Progressive Montreal phenotype, RBWH cohort

The same analysis, using the Montreal classification system, may be applied to our cohort. (see figure 7.1) When this is done it demonstrates that in our cohort long term disease outcome appears similar to that seen in the *Saint-Antoine* cohort of Cosnes et al: a progression from inflammatory to stricturing to penetrating phenotype. It became apparent looking at our dataset that as time passed stricturing complications occurred more commonly than penetrating complications. This led to consideration as to why the Montreal classification system represents disease progression in our cohort in this way.

The Montreal classification system classification describes disease progression from inflammatory, to stricturing, to penetrating because it is defined to be *hierarchical* and *irreversible*. A patient with a penetrating complication at any timepoint in his or her disease course is classified as penetrating from that time forward, regardless of what happens subsequently.

We have considered classification of stenosing and penetrating complications in our cohort in a slightly different way, removing the *hierarchical* and *irreversible* aspects of the Montreal classification system as follows.

1. Classification at any one time-point is calculated from all complications that occur in the 3 years prior to that timepoint.

2. Classification is not hierarchical. This means that four levels of classification are necessary: Inflammatory, Stricturing, Penetrating, Stricturing *and* Penetrating.

Using this classification, a *rolling Montreal phenotype*, it is evident that the most commonly occurring complication in our cohort over time is stricture, and that this is true regardless of what complications are observed in the first year of disease course. (see figures 7.2, 7.3, 7.4, 7.5) The large change in all classification at three years is due to the high proportion of patients who suffer a complication at diagnosis, and then no further complication following this. Because of *rolling Montreal phenotype* definition, this is observed 3 years after their last complication - which was at or around the time of diagnosis.

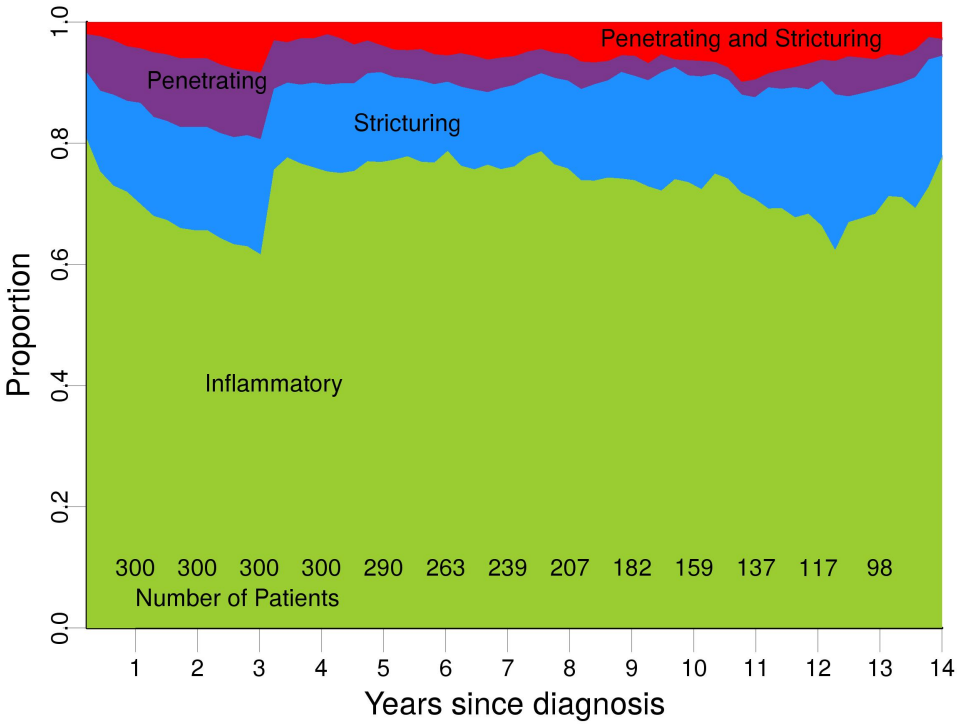


**Figure 7.2**
Rolling Montreal phenotype, RBWH cohort

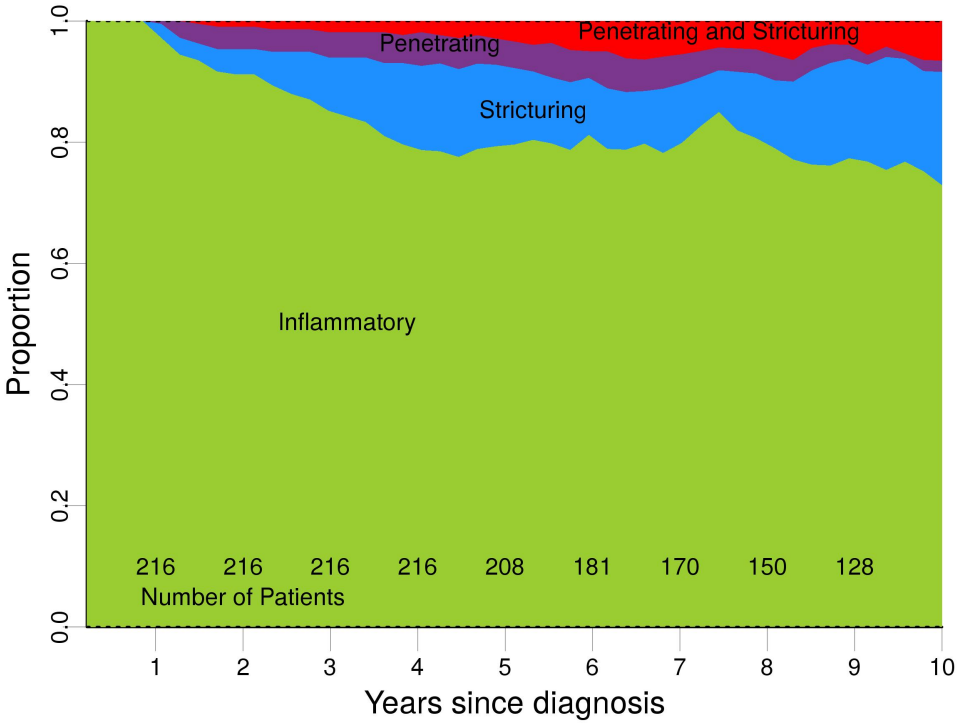


**Figure 7.3**
Rolling Montreal phenotype, B1 phenotype within first year only, RBWH cohort

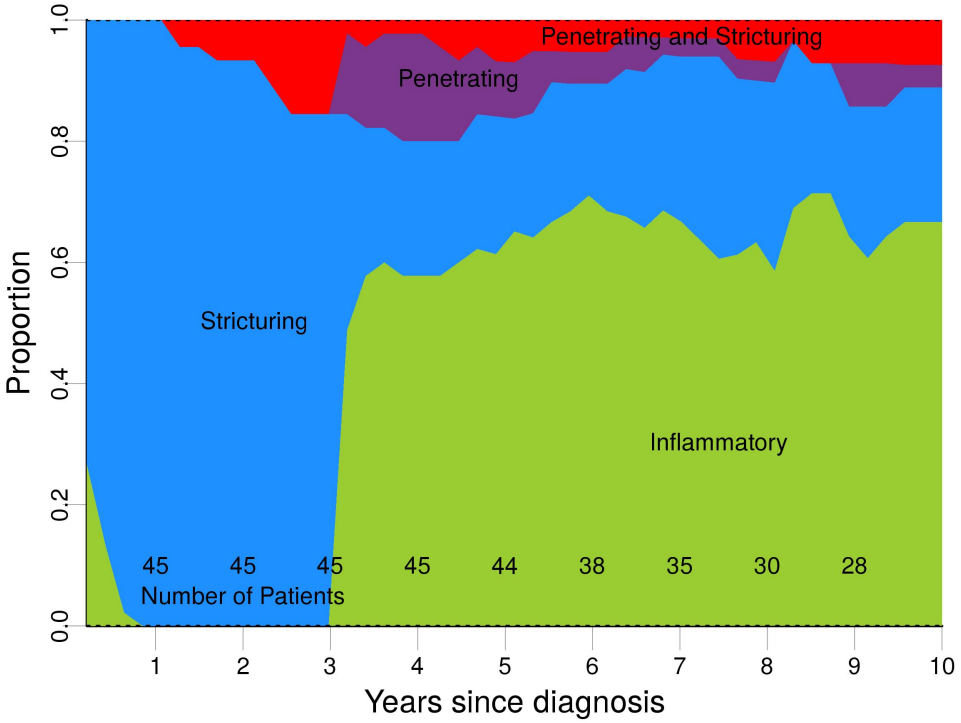


**Figure 7.4**
Rolling Montreal phenotype, B2 phenotype within first year only, RBWH cohort

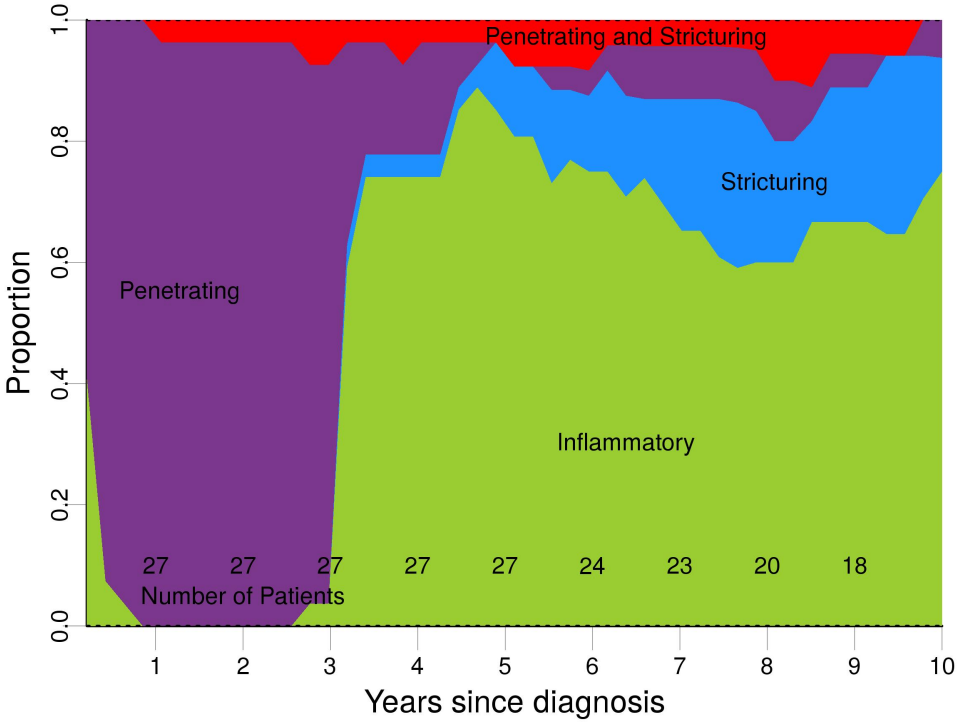


**Figure 7.5**
Rolling Montreal phenotype, B3 phenotype within first year only, RBWH cohort

## 7.2. Prediagnosis inflammatory signature

After matching laboratory data to our cohort we observed that many of the laboratory tests obtained were taken before patients were diagnosed with Crohn's disease. There are published data suggesting that delay from reported onset of symptoms to diagnosis of Crohn's disease is in the order of 9 months.(Schoepfer, Dehlavi, et al. 2013) Our laboratory data should provide a more objective marker of disease activity prior to diagnosis. Is there a signature in blood testing performed on patients prior to their diagnosis of Crohn's disease, and how early is it detectable?

This data was analyzed comparing results taken in the two years leading up to diagnosis, considering results taken between two to three years prior to diagnosis as a baseline. Date of diagnosis was assigned when the treating physician considered the patient to have Crohn's disease and initiated treatment. This was usually when endoscopy or imaging was performed to confirm the presence of bowel inflammation in the presence of chronic symptoms.

Many patients did not have blood testing performed prior to diagnosis. The number of test results available for analysis are less than those available after diagnosis.

In this cohort there is a clear signal present in most blood tests that becomes apparent 12 months prior to the date of diagnosis. Haemoglobin level and albumin level begin to drop at this point, while platelet count, albumin level ESR and CRP begin to rise. (see figures 7.6, 7.7, 7.8, 7.10, 7.9)

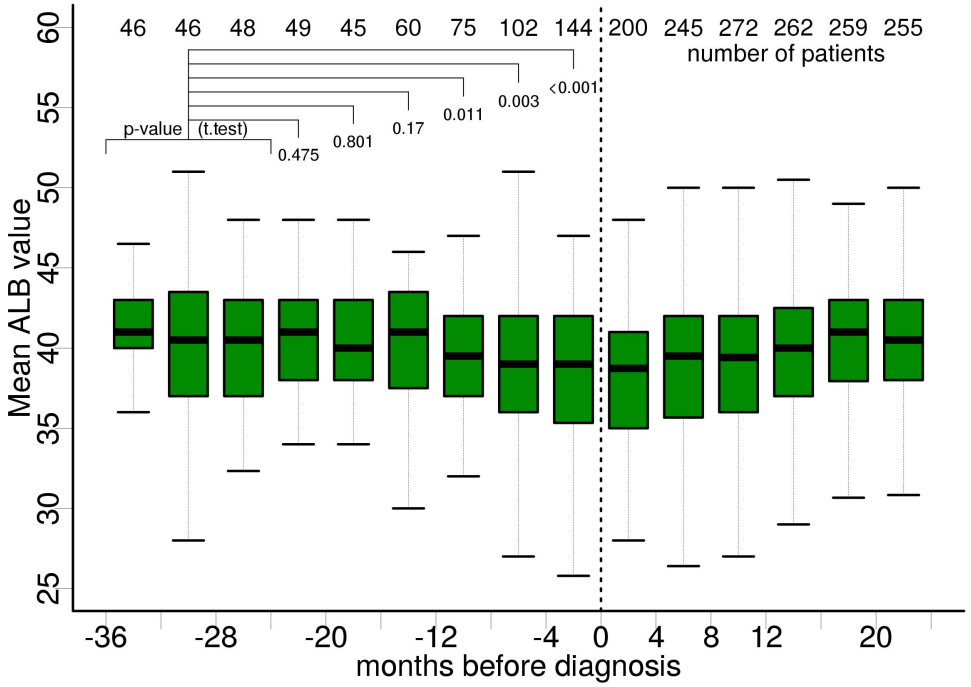


**Figure 7.6**
Prediagnosis laboratory signature: albumin

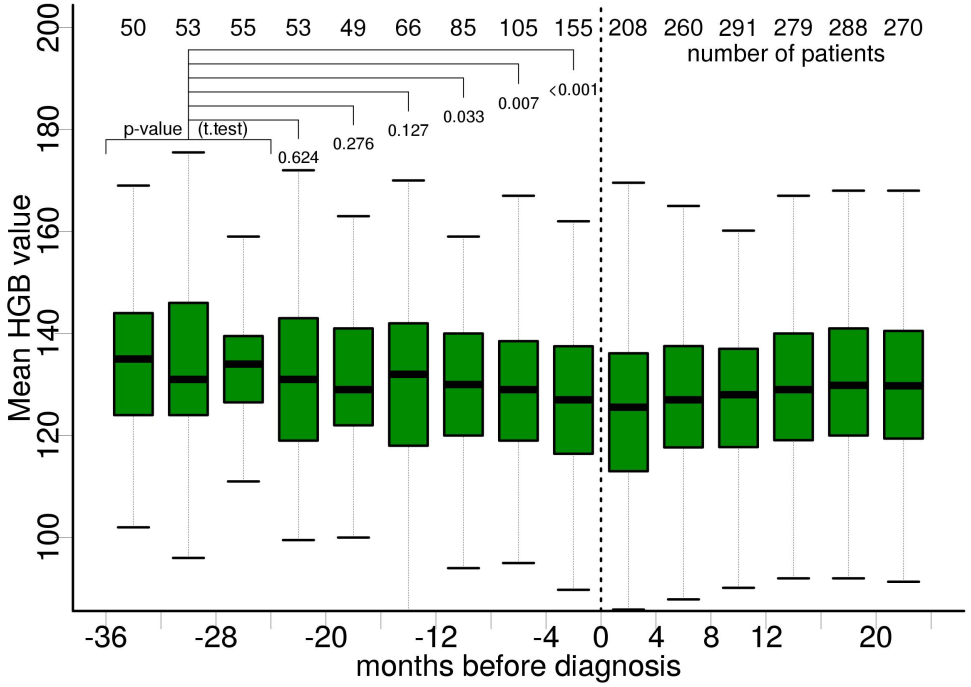


**Figure 7.7**
Prediagnosis laboratory signature: haemoglobin level

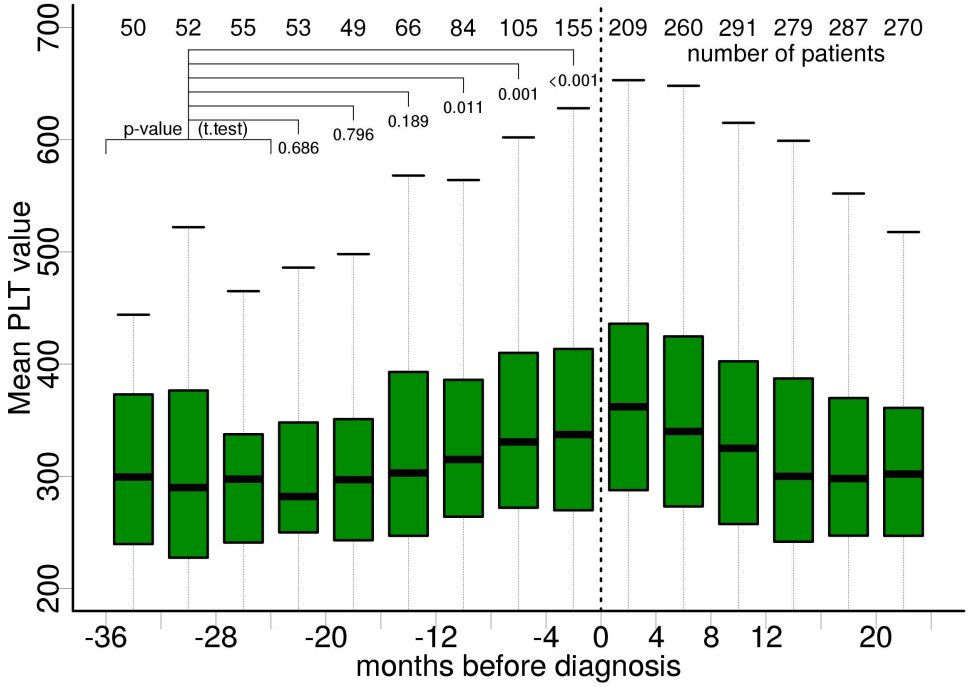


**Figure 7.8**
Prediagnosis laboratory signature: platelet count

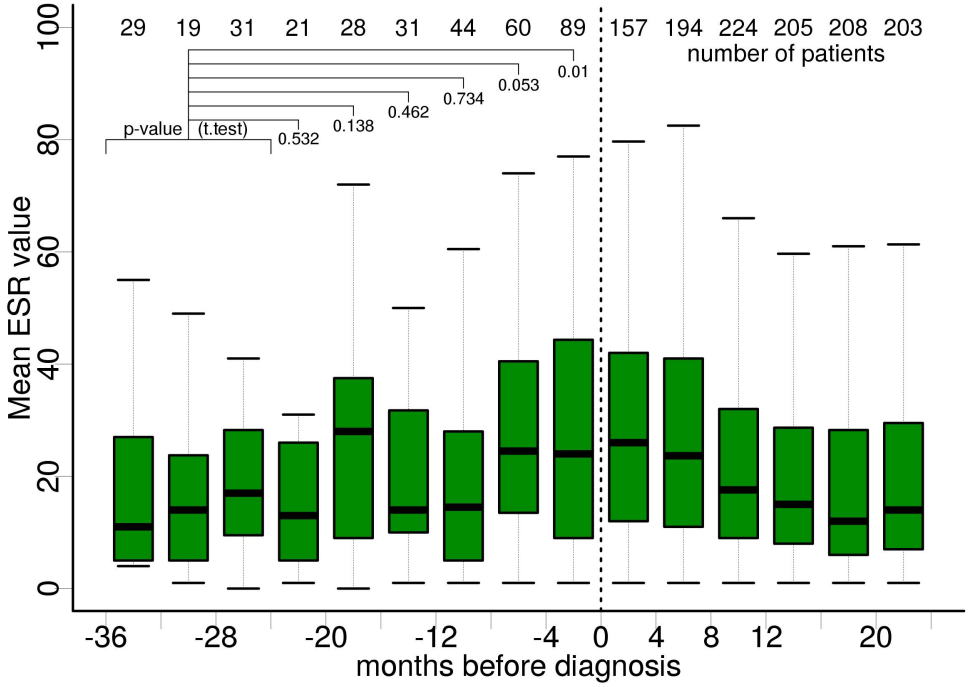


**Figure 7.9**
Prediagnosis laboratory signature: ESR

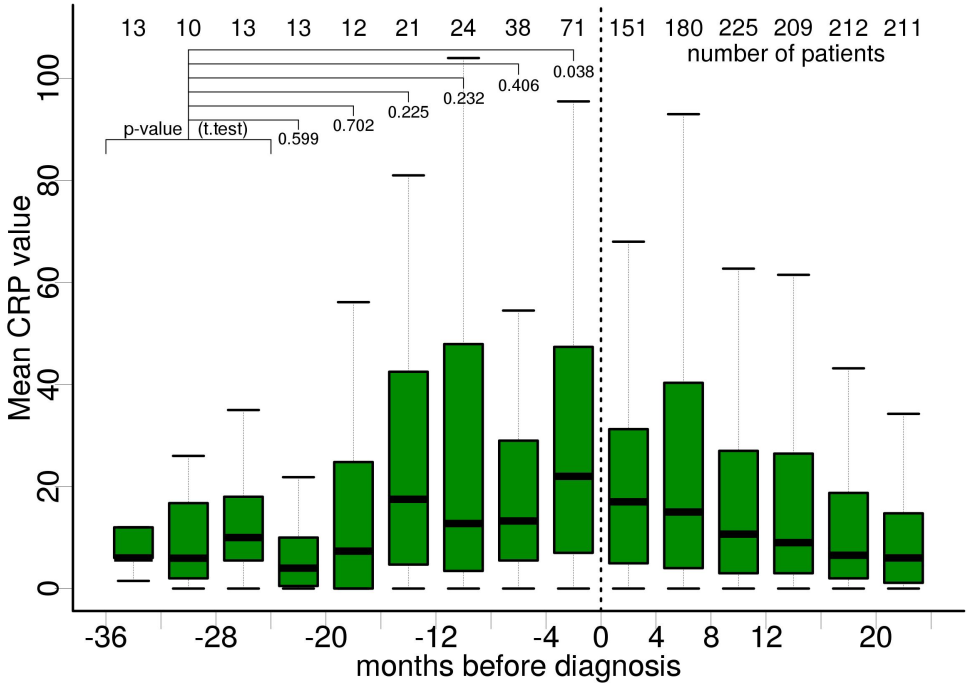


**Figure 7.10**
Prediagnosis laboratory signature: CRP

Although these data are not useful for individual patients in the years leading up to diagnosis, they do provide useful insight into prediagnostic inflammatory bowel disease. These results agree with published data showing that clinical symptoms are present for 9 months prior to diagnosis in patients with Crohn's disease. (Schoepfer, Dehlavi, et al. 2013) They add additional weight to this finding in that they are objective results, and are not subject to subjective patient recall of duration of symptoms.

## 7.3. Delay to meeting formal diagnostic criteria in Crohn's disease

There is a lack of consensus as to what criteria should be used to assign a patient a diagnosis of Crohn's disease. We feel our detailed longitudinal data provides some insight into the process of diagnosing Crohn's disease, through analysis of the timecourse to meeting various diagnostic criteria.

What is Crohn's disease? There is consensus that Crohn's disease is an (often granulomatous) transmural chronic inflammatory condition of the human gastrointestinal tract. Involvement may include segments of the gastrointestinal tract from the mouth to the anus, and histologically it is characterized by submucosal inflammation, fibrosis and ulceration. In its original description Crohn's disease was considered a chronic inflammatory condition of the terminal ileum only. (Crohn BB, Ginzburg L, and Oppenheimer GD 1932, see figure 7.11) In the 1960's chronic colonic inflammation with transmural features or presence of granulomata on histological specimen also came to be considered Crohn's disease.(Lockhart-Mummery and Morson 1960) Chronic inflammation of the oesophagus, stomach, duodenum, jejunum and orobuccal mucosa also came to be recognized as part of the spectrum of Crohn's disease, as they were often accompanied by disease typical of Crohn's disease in the ileum and colon. Formal diagnostic criteria were lacking until *Lennard-Jones* proposed standardized criteria with which to assign a diagnosis of Crohn's disease. (Lennard-Jones 1989, see table 3)

The European Crohn's and Colitis Organization (ECCO) published a consensus statement which includes a statement on the diagnosis of Crohn's disease. (Van Assche et al. 2010) These diagnostic criteria acknowledged that the *Lennard-Jones* criteria often depend on a transmural surgical specimen to confirm a diagnosis of Crohn's disease. The ECCO diagnostic criteria attempt to provide clinical guidance to allow a diagnosis of Crohn's disease in the absence of a transmural surgical

> We propose to describe, in its pathologic and clinical details, a disease of the terminal ileum, affecting mainly young adults, characterized by a subacute or chronic necrotizing and cicatrizing inflammation. The ulceration of the mucosa is accompanied by a disproportionate connective tissue reaction of the remaining walls of the involved intestine, a process which frequently leads to stenosis of the lumen of the intestine, associated with the formation of multiple fistulas.The disease is clinically featured by symptoms that resemble those of ulcerative colitis, namely, fever, diarrhea and emaciation, leading eventually to an obstruction of the small intestine; the constant occurrence of a mass in the right iliac fossa usually requires surgical intervention (resection). The terminal ileum is alone involved. The process begins abruptly at and involves the ileocaecal valve in its maximal intensity, tapering off gradually as it ascends the ileum orally for from 8 to 12 inches.....

**Figure 7.11**
From *Regional ileitis: A pathologic and clinical entity* Crohn et al, Journal of the American Medical Association, 1932

specimen. However, in doing this they are less precise in how Crohn's disease is defined.

Research into all aspects of Crohn's disease relies on a precise definition of the condition. Lack of a precise definition leads to heterogeneity in research cohorts of patients with Crohn's disease, and less certainty that observations made in one cohort of patients with Crohn's disease are applicable to another cohort. Variation between research cohorts in the proportion of patients with disease limited to the colon could be considered one marker of this heterogeneity. In published cohorts this proportion varies between 20% - 50%. (Loly, Belaiche, and Louis 2008; Tarrant et al. 2008; Magro et al. 2014; Lapidus 2006) We believe this heterogeneity results from variation in interpretation of diagnostic criteria for Crohn's disease.

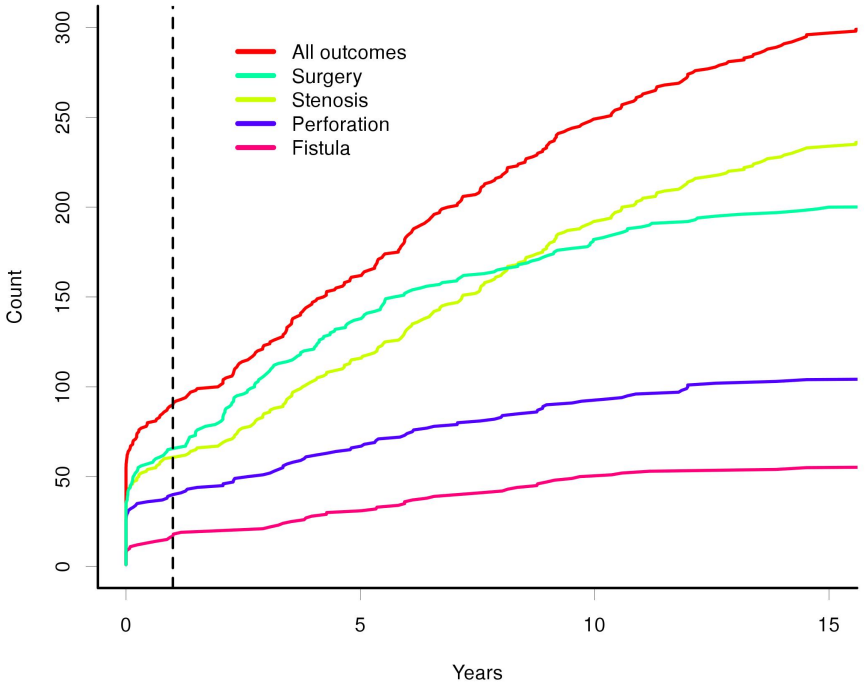


**Figure 7.12**
Cumulative incidence of complications, RBWH cohort
n=302

The current paradigm of medical management of Crohn's disease is that chronic bowel inflammation leads to irreversible tissue damage, and that therapy to consistently suppress inflammation will prevent tissue damage. This approach to the management of Crohn's disease requires early implementation of therapy, before the development of irreversible tissue damage. The provision of early therapy depends on early diagnosis. Many of the complications seen in Crohn's disease occur within the first 6 months of diagnosis, as was observed in our cohort (see figure 7.12). We believe that neither the *Lennard-Jones* criteria nor the *ECCO Consensus Criteria* are well designed to progress research in assessing the impact of early medical intervention on the natural history of Crohn's disease. The *Lennard-Jones* criteria depend too heavily on the identification of evidence of a transmural disease process to assign a diagnosis (a fistula, a stenosis or transmural histology obtained from a surgical specimen). For many patients, by the time they meet *Lennard-Jones* criteria for a diagnosis of Crohn's disease, it is too late to implement therapy to try to prevent these complications. In contrast, the *ECCO Consensus Criteria* do allow assignment of a diagnosis of Crohn's disease early in a patient's disease

course, without a surgical specimen. However, they are open to interpretation, and if used to assign the diagnosis of Crohn's disease in research cohorts, will lead to further heterogeneity in patient populations.

In order to study the early disease course of Crohn's disease, (natural history, impact of medical or surgical therapy) diagnostic criteria for Crohn's disease must be clearly defined without requiring evidence of a transmural disease process. Altering the diagnostic criteria for Crohn's disease in this way would almost certainly assign a diagnosis of Crohn's disease to patients who by previous criteria would not have received a diagnosis. However, research populations need to be transparently defined in order to clearly assess natural history and response to therapy early in their disease course.

Has there been a delay to meeting either *Lennard-Jones* criteria or *ECCO Consensus Criteria* for a diagnosis of Crohn's disease in our cohort? This information will provide some insight into how current diagnostic criteria perform in the early identification of patients with Crohn's disease.

We consider that there are two main groups of patients who often do not meet *Lennard-Jones* diagnostic criteria for Crohn's disease early in their disease course. One group are those patients with non-granulomatous disease isolated to the colon without evidence of transmural complication. These patients are not able to be clearly distinguished from patients with ulcerative colitis. The second group are those with non-granulomatous disease isolated to the terminal ileum, without evidence of transmural complication. For diagnostic purposes both groups need a transmural histological specimen (a surgical specimen) to confirm or refute the presence of submucosal ulceration or fibrosis. However, this information is not available until a bowel resection is indicated, and performed. In our experience the first group of patients will usually be advised to take appropriate therapy whether they are considered to have Crohn's disease, inflammatory bowel disease type unspecified (IBD-TU) or ulcerative colitis. We observe that the second group

of patients have a less clear disease trajectory, and physicians may be reluctant to assign a diagnosis of Crohn's disease and commit to immunomodulatory therapy (and its side effects).

### 7.3.1. A brief description of methods

For this analysis the gold standard for a diagnosis of Crohn's disease was considered to be meeting of the *Lennard-Jones* criteria within 5 years of diagnosis. We restricted the analysis to these patients, and observed what at what time diagnostic criteria were met. The date of diagnosis was the date on which the treating physician felt a diagnosis of Crohn's disease had been met, and therapy to treat Crohn's disease was initiated. This was usually when mucosal tissue was available for histological examination after ileocolonoscopy. The *Lennard-Jones* criteria were considered met when 3 of the following criteria were present (2 if one was granuloma): typical location, discontinuous macroscopic disease, transmural ulceration, transmural fibrosis, lymphocytic aggregation, and presence of granulomas. The *ECCO Consensus Criteria* do not provide strict diagnostic criteria, and for the purpose of this analysis only mucosal histological criteria were analyzed. The *ECCO histological criteria* were considered met when 3 of the following criteria (2 if one was granuloma) were present on mucosal biopsies or surgical specimens: lymphocytic infiltrate, crypt architectural distortion, irregular villous architecture (ileum), crypt abscesses, submucosal fibrosis, fissuring ulceration, and presence of granulomas.

### 7.3.2. Results

This analysis demonstrates that 25% of the RBWH longitudinal cohort did not meet the Lennard-Jones criteria for a diagnosis of Crohn's disease within one year of diagnosis, despite meeting these criteria over the next four years.(see figure 7.13) This observation confirms that the Lennard-Jones criteria are too strict to allow

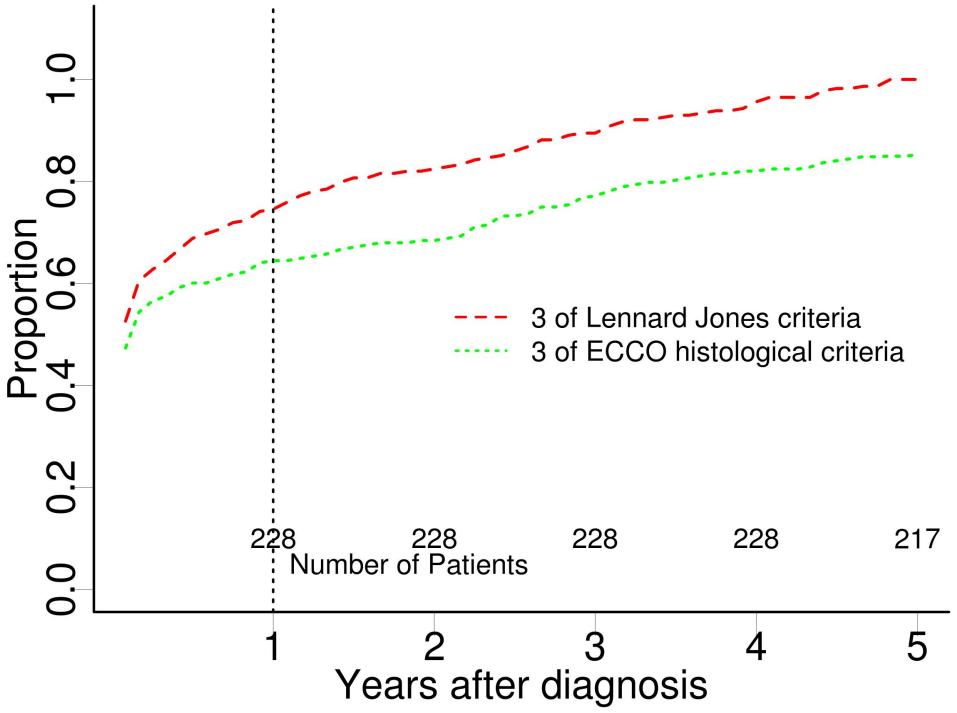


**Figure 7.13**
Delay to fulfill Lennard-Jones criteria and ECCO histological criteria.
Analysis limited to patients who fulfilled the Lennard-Jones criteria within 5 years.
ECCO = European Crohn's and Colitis Organization

early identification of a significant proportion of patients who definitely evolve to have a transmural chronic inflammatory bowel condition. Further exploration of these data should yield some insight into the characteristics of patients who don't meet the Lennard-Jones criteria early in their disease course. We may also demonstrate how they may be identified through characteristics aside from the Lennard-Jones criteria.

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# Classification Systems in Crohn's disease

| *Variable* | *0* | *1* | *2* | *3* |
|---|---|---|---|---|
| *Size of ulcers* | None | Aphthous ulcers ( $0.1 < \theta < 0.5$ cm) | Large ulcers ( $0.5 < \theta < 2$ cm) | Very large ulcers ($\theta > 2$ cm) |
| *Ulcerated surface* | None | <10% | 10-30% | >30% |
| *Affected surface* | Unaffected segment | <50% | 50-75% | >75% |
| *Presence of narrowings* | None | Single, can be passed | Multiple, can be passed | Cannot be passed |

**Table 1**
Simple endoscopic score - Crohn's disease
$\theta$ = *diameter*
*A score is calculated for each of five segments of the bowel: rectum, left colon, transverse colon, right colon, ileum.*
*Total score is the sum of 5 segmental scores.*

| | |
|---|---|
| Number of liquid or soft stools, each day, for seven days | x 2 |
| Abdominal pain ☆ | x 5 |
| General well being † | x 7 |
| Presence of complications ⋆ | x 20 |
| Taking medication to reduce diarrhoea | x 30 |
| Presence of an abdominal mass ✈ | x 10 |
| Haematocrit of <0.47 in men and <0.42 in women ✝ | x 6 |
| Percentage deviation from standard weight ✢ | x 1 |

**Table 2**
Crohn's disease activity index. (Best et al. 1976)
☆ *graded from 0-3 on severity each day, for seven days*
† *subjectively assessed from 0 (well) to 4 (terrible) each day, for seven days*
⋆ *Score 20 for each complication: the presence of joint pains (arthralgia), iritis, erythema nodosum, pyoderma gangrenosum, aphthous ulcers, anal fissure, perianal fistula or abscess, other fistula, fever during the previous week.*
✈ *0 none, 2 questionable, 5 definite*
✝ *× 6 for each 0.01 below cut-off value*

*Remission is a score <150*

| | |
|---|---|
| **Exclusions** | |
| | Infection |
| | Ischaemia |
| | Malignancy |
| **Inclusions** | |
| *Location includes* | |
| | Lip or buccal mucosa |
| | Pyloro-duodenal disease |
| | Small bowel disease |
| | Chronic anal lesion |
| *Discontinuous* | |
| | Lesions separated by normal mucosa |
| *Lymphoid* | |
| | Presence of lymphoid |
| | aggregates on biopsy |
| *Transmural* | |
| | Fissuring ulcers |
| | Abscess |
| | Fistula |
| *Mucin* | |
| | Retention of colonic mucin on biopsy |
| *Granulomata* | |
| | Distinguished from caseating granulomata |

**Table 3**
Lennard-Jones criteria for diagnosis of Crohn's disease. (Lennard-Jones 1989)
*Meeting 3 of inclusion features is suggested to confirm a diagnosis*

| | |
|---|---|
| *Age at diagnosis* | |
| A1 | <16 years |
| A2 | between 17 and 40 years |
| A3 | >40 years |
| *Location* | |
| L1 | Terminal Ileum |
| L2 | Colon |
| L3 | Ileocolon |
| L4 | Isolated upper gastrointestinal |
| *Behaviour* | |
| B1 | Non-stricturing, Non-penetrating |
| B2 | Stricturing |
| B3 | Penetrating |
| *Modifiers* | |
| p | Perianal disease modifier |
| L4 | Upper gastrointestinal involvement concurrent with other disease location |

**Table 4**
Montreal classification. (Satsangi et al. 2006)

| Organ | Investigational Method | n[a] | Segment | Grade 1 | Grade 2 | Grade 3 |
|---|---|---|---|---|---|---|
| **Surgical interventions** | | | | | | |
| Upper tract | | 3 | Esophagus Stomach Duodenum | — | Bypass diversion or stricturoplasty | Resection |
| Small bowel | | 20 | Each 20-cm segment | — | Bypass diversion or stricturoplasty | Resection |
| Colon/Rectum | | 6 | Each segment | — | Stomy, bypass diversion or stricturoplasty | Resection |
| Anus | | 1 | Anus | Reconstruction procedure, flap, coring out fistula track or laying open of fistula | Major surgery leading to substantial sphincter damage[b]<br>Temporary diversion | Definitive diversion<br>Proctectomy |
| **Stricturing lesions** | | | | | | |
| Upper tract | Endoscopy | 3 | Esophagus Stomach Duodenum | — | Lumen narrowing, passable | Stricture, nonpassable |
| | MRI or CT | 2 | Stomach Duodenum | Wall thickening <3 mm or segmental enhancement without prestenotic dilatation | Wall thickening $\geq$ 3mm or mural stratification without prestenotic dilatation | Stricture with prestenotic dilatation |
| Small bowel | MRI or CT | 20 | Each 20-cm segment | Wall thickening <3 mm or segmental enhancement without prestenotic dilatation | Wall thickening $\geq$ 3 mm or mural stratification without prestenotic dilatation | Stricture with prestenotic dilatation |
| Colon/Rectum | Colonoscopy | 6 | Each segment | — | Lumen narrowing, passable | Stricture, non passable |
| | MRI or CT | 6 | Each segment | Wall thickening <3 mm or segmental enhancement without prestenotic | Wall thickening $\geq$3 mm or mural stratification without prestenotic dilatation or | Stricture with prestenotic dilatation or >50% of the lumen |

| | | | | | | |
|---|---|---|---|---|---|---|
| | | | | dilatation | <50% of the lumen | |
| Anus | Clinical examination | 1 | Anus | Mild stricture | Frank stricture, passable | Frank stricture, nonpassable |
| **Penetrating lesions** | | | | | | |
| Upper tract | Endoscopy | 3 | Esophagus Stomach Duodenum | Superficial ulceration | Deep ulceration | Fistula |
| | MRI or CT | 2 | Stomach Duodenum | — | Deep transmural ulceration | Phlegmon or any type of fistula |
| Small bowel | MRI or CT | 20 | One 20-cm segment | — | Deep transmural ulceration | Phlegmon or any type of fistula |
| Colon/rectum | Colonoscopy | 6 | Each segment | Superficial ulceration | Deep ulceration | Fistula |
| | MRI or CT of fistula | 6 | Each segment | — | Transmural ulceration | Phlegmon or any type |
| Anus examination | Clinical extensive anal and perianal tissue destruction | 1 | Anus | Anal ulceration | Multiple fistulae | Multiple fistulae with |
| | MRI or CT[c] | 1 | Anus | Simple fistula[d] | Branching fistula, multiple fistulae, or any type of abscess >1 cm | Extensive anal and perianal suppuration, horseshoe abscess, or fistula(e) involving or extending above the levator plate |

**Table 5**
The Lémann score; proposed severity score of irreversible tissue damage in patients with Crohn's disease small bowel. (Pariente, Mary, et al. 2015) [a]Number of segments.
[b]Division of the internal anal sphincter, external anal sphincter, or both for half or more of the length of the anal canal.
[c]Only in case of abnormality at clinical examination.
[d]Fistula extending from the anal canal to the perianal skin, but involving only the lowermost, or none, of the anal sphincter muscles, and without any secondary tracks).

# Coding definitions

Many variables used in these analyses were defined using automated algorithms which took raw data from a database recording objective data, the *longitudinal Crohn's disease* database. This chapter summarizes these definitions which are written in the statistical programming language "R". The inclusion of this code is intended to provide a precise definition of how variables were defined.

## .1. Low level functions

These functions were defined to allow manipulation of observational data from the *longitudinal Crohn's disease* database. They are called in higher level functions which define outcomes such as perianal fistula formation, or identification of bowel stenosis.

```
1 fun_binary<-function(vector){
2 ##function to convert all NA strings to 0, and all numbers to 0 or 1, to allow
      combination of extent vectors. (0 or NA -> no involvement, 1 or greater ->
      involvement)
3 vector<-ifelse(is.na(vector),0,
4 ifelse(vector==0,0,1))
5 return(vector)
6 }
```

**Listing 1**

Binary Evaluator

```
1 ##define 'sub_code function'
2 sub_code<-function(path,file){
3 ##'path' is PATH eg "home/james/cd/"
```

```
##'file' is filename to be run. It must be a .R file. The output will be saved as
     .Rout in the same folder.
sink(paste(path,file,".Rout",sep=""))
source(paste(path,file,".R",sep=""),echo=TRUE,max.deparse.length=10000)
sink()
}
```

**Listing 2**

Sub code routine.

This code calls subroutines into a master coding script. It allows simplified and repetitive use of lower level functions and code snippetts in different analyses.

```
fun_make_relational<-function(a,b){
##function for making a relational database for one investigation or set of
    datapoints (for example surgeries, or abdominal radiology)
##a=name of field to be used to grep columns (eg "^Surgery", or "RadAbdo", or "^
    Colo", or "^PAClinical")
##b=name of database from which fields will be taken
##colnames will be taken from cd database
fields<-colnames(b)[grep(a,colnames(b))]
names<-fields[grep("[a-zA-Z]1$|[a-zA-Z]1Date$",fields)]
names<-gsub("[0-9]","",names)
names<-c("id",names)
aa<-rep(NA,length(names))
start<-min(grep("1",fields))
if(start>1){
fields<-fields[-(1:(start-1))]
}
rep<-min(grep("2",fields))-1
rep2<-length(fields)/rep
for(i in 1:rep2){
aaa<-b[,c("id", fields[((i-1)*(rep)+1):(i*rep)])]
colnames(aaa)<-names
aa<-rbind(aa,aaa)
}
out<-aa
out<-subset(out,!is.na(out[,2]))
if(TRUE%in%(grepl("DateStart", names))){
out<-out[order(out[,grep("DateStart",names)],na.last=TRUE),]
}else if(TRUE%in%(grepl("Date", names))){
out<-out[order(out[,grep("Date",names)],na.last=TRUE),]
} else if (TRUE%in%(grepl("^DCW", names))) {
out<-out[order(out[,grep("DCW",names)],na.last=TRUE),]
}
out<-as.data.frame(out)
return(out)
}
```

**Listing 3**

Relational Conversion of Longitudinal Database.

The *longitudinal Crohn's Disease* database is not in a relational format. This following function converts data into relational format to allow further analysis to occur with a simplified, relational data structure.

```
fun_numericFactor2text<-function(a,b,c){
##function to convert to text description of levels
#a represents string to convert eg 'indicationsurgerystring' (double quotes)
#b represents field on which conversion will occur eg 'SurgeryInd' (double quotes)
#c represents database eg 'cd' (no quotes)
test<-factor(c[,b])
test<-factor(c[,b],levels=sort(as.numeric(levels(test))))
levs<-levels(test)
n<-grep(a,string)
levs2<-get(string[n])
levs3<-levs2[!levs2%in%levs]
levels(test)<-c(levs,levs3)
test<-factor(test,levels=levs2)
levels(test)<-get(string_word[n])
return(test)
}
```

**Listing 4**

Conversion of Numeric coding to descriptive coding.

This function converts from numeric to descriptive strings, the conversion vectors are contained in the master coding document "CD_coding.tex". The relationship between numbers and descriptive text is contained in the chapter "Definition of Coding Strings" in the coding manual. Information coded by descriptive strings is useful for production of graphs or tables.

```
fun_numericFactor2textstring<-function(a,b,c){
##function to convert to text description of levels, for string fields
#a represents string to convert eg 'indicationsurgerystring' (double quotes)
#b represents field on which conversion will occur eg 'SurgeryInd' (double quotes)
#c represents database eg 'cd' (no quotes)
test<-strsplit(c[,b],split=",")
test<-list2df(test)
for (i in 1:length(colnames(test))){
test[,i]<-factor(test[,i])
test[,i]<-factor(test[,i],levels=sort(as.numeric(levels(test[,i]))))
```

```
levs<-levels(test[,i])
n<-grep(a,string)
levs2<-get(string[n])
levs3<-levs2[!levs2%in%levs]
levels(test[,i])<-c(levs,levs3)
test[,i]<-factor(test[,i],levels=levs2)
levels(test[,i])<-get(string_word[n])
}
return(test)
}
```

**Listing 5**

Conversion of Numeric coding to descriptive coding: for string variables

## .1.1. Grep functions

These functions are used to extract data from string variables. For example, on a colonoscopy the extent of bowel involvement from anus to splenic flexure would be coded *"9,10,11"* in the field *ColoExtent*. The following functions allow manipulation of these strings to search for included coded integers. This allows a user to ask questions such as "Is there ileal involvement on colonoscopy?", or "Are there granulomas on histology taken from colonic (not ileal) biopsy?"

## .1.2. Strings for defining extent of bowel

```
##define grepstrings for confirming extent
##as vectors
grepcolo<-grepstringvector(c("8","9","10","11"))
grepsmallbowel<-grepstringvector(c("6","5","4"))
grepupper<-grepstringvector(c("1","2","3"))
grepcaecum<-grepstringvector(c("7"))
grepbowel<-grepstringvector(c("4","5","6","7","8","9","10","11"))
grepstomachduodenum<-grepstringvector(c("2","3"))
grepjejunum<-grepstringvector(c("4"))
grepileum<-grepstringvector(c("5","6"))
##as single string
grephisto.upper<-grepstring(c("4","4"))
grephisto<-grepstring(c("1","2","3","4","5","6","7"))
```

```
grepfistula<-grepstring(c("1","2","3","4","5","6","7","8","9","10","11","12","20"
    ))
grepabcess<-grepstring(c("1","2","3","4","5","6","7"))
grepabcessSurg<-grepstring(c("2"))
grepabcessRad<-grepstring(c("1","2"))
grepPAclinical<-grepstring(c("2","5"))
grepPAparks<-grepstring(c("3","4"))
grepPAcomplex<-grepstring(c("1","2","3"))
grepDilation<-"^[3-9][.,]+.*$|^10.*$|^[3-9]$"
grepcolostring<-grepstring(c("8","9","10","11"))
grepsmallbowelstring<-grepstring(c("6","5","4"))
grepproxileum<-grepstring(c("5","4"))
grepgastrojejunostomy<-grepstring(c("2","3"))
```

**Listing 6**

Definition of strings for defining particular bowel segments, or combinations of other codes

## .1.3. Calculation of bowel extent

These functions are used to calculate extent of disease, or of an abnormality (for eg. a fistula) on any modality. *fun_extent* calculates simple extent of disease observed on one type of procedure. How segments of bowel have been coded as involved or uninvolved differs by modality (eg colonoscopy vs. radiological procedure) and are documented in the coding manual. *fun_extent_double* allows more detailed analysis, for example for colonscopy procedures which segments are involved with granulomas present on histology, or which segments are involved microscopically but not macroscopically.

```
fun_extentMaster<-function(...){
##function to combine modalities for extent (eg: surgery, radiology and
    colonoscopy)
list<-lapply(list(...), fun_binary)
df<-lapply(list,as.data.frame)
df<-t(ldply(list))
out<-unlist(rowSums(df,na.rm=TRUE))
return(out)
}
```

**Listing 7**

Master function to combine extent calculated from different modalities

```
fun_extent<-function(modality,dateModality,extentfield,location,dataframe,date,
    add){
##function to determine extent as observed by a modality (either surgery,
    radiology or colonoscopy. Output of this function will be combined at a later
     point.
##modality is name of relational dataframe eg < Colo > or < RadAbdo >
##note that limiting of dataset to wanted investigations (eg only include
    ileocolonoscopy and not flexible sigmoidoscopy) must be performed before
    passing the dataframe to this function.
##dateModality is the datefield in < modality >
##extentfield is the field in which extent is recorded eg "RadAbdoExtent" or "
    ColoExtent" or "ColoHistoMicroExtent"
##location is location of interest eg colon, or ileum, or caecum: location is
    either referred to as < grepcolo > or < grepileum >
##dataframe is dataframe of patients being examined eg < cd > or < PRIME >
##date is a date vector in < dataframe > which is the time-limit of this
    definiton. Eg "DateDiag1" (date diagnosis) or "LstSeen" which is date of last
     follow-up.
##add is the time (in days) after < date > which serves as a cutoff - eg.
    investigations performed within 180 days of diagnosis.
test<-!is.na(as.Date(modality[,dateModality]))
tempMod<-subset(modality,test)
test2<-!is.na(as.Date(dataframe[,date]))
tempDF<-subset(dataframe,test2)
l<-length(tempDF[,1])
out2<-rep(NA,l)
out3<-rep(NA,length(dataframe[,1]))
for(i in 1:l){
out<-as.list(rep(NA,length(location)))
test3<-tempMod$id==tempDF$id[i] & as.Date(tempMod[,dateModality])<= (as.Date(
    tempDF[i,date])+add)
temp<-subset(tempMod,test3)
if(length(temp[,1])>0){
for(j in 1:length(location)){
for(k in 1:length(temp[,1])){
temp2<-grepl(location[j],temp[k,extentfield])
out[j]<-paste(out[j],temp2)
}
out[j]<-grepl("TRUE",out[j])
}
out2[i]<-sum(unlist(out),na.rm=TRUE)
}
}
print(out2)
for(m in 1:length(dataframe[,1])){
if(dataframe$id[m]%in%tempDF$id){
out3[m]<-out2[tempDF$id==dataframe$id[m]]
```

```
}
}
return(out3)
}
```

**Listing 8**

Calculation of extent of an abnormality on a single modality

```
fun_extent_double<-function(modality,dateModality,extentfield,location,
    secondfield,secondgrepstring,dataframe,date,add){
##function; same as fun_extent except:
##secondfield is the field in which certain criteria need to be met (eg.
    histological findings) before involvement is considered to be present.
    Examples for this field are recorded eg "RadAbdoExtent" or "ColoExtent" or "
    ColoHistoMicroCoded"
##secondgrepstring;  referred to as < grephisto > or similar. Must be a single
    string, and not a vector of strings.
test<-!is.na(as.Date(modality[,dateModality]))
tempMod<-subset(modality,test)
test2<-!is.na(as.Date(dataframe[,date]))
tempDF<-subset(dataframe,test2)
l<-length(tempDF[,1])
out2<-rep(NA,l)
out3<-rep(NA,length(dataframe[,1]))
for(i in 1:l){
out<-as.list(rep(NA,length(location)))
test3<-tempMod$id==tempDF$id[i] & as.Date(tempMod[,dateModality])<= (as.Date(
    tempDF[i,date])+add)
temp<-subset(tempMod,test3)
if(length(temp[,1])>0){
for(j in 1:length(location)){
for(k in 1:length(temp[,1])){
temp2<-grepl(location[j],temp[k,extentfield]) & grepl(secondgrepstring,temp[k,
    secondfield])
out[j]<-paste(out[j],temp2)
}
out[j]<-grepl("TRUE",out[j])
}
out2[i]<-sum(unlist(out),na.rm=TRUE)
}
}
print(out2)
for(m in 1:length(dataframe[,1])){
if(dataframe$id[m]%in%tempDF$id){
out3[m]<-out2[tempDF$id==dataframe$id[m]]
}
}
return(out3)
```

```
}
```

**Listing 9**

Calculation of extent of an abnormality on a single modality, with additional restricting criteria

## .1.4. Medication functions

This function defines medication use as the proportion of time taking the medication in the observation period preceding an outcome of interest. For example it could state that azathioprine was taken for 46% of the time during the observation period. It also generates the duration of the observation period in days.

```
fun_medication<-function(df.med,type,values,datestart,datestop,df.outcome){
##function calculates a representative number of exposure to a medication - 1 represents full exposure over the observation period, 0 represents no exposure.
##for eg: fun_medication(IS,c(1,2,6),"ISDateStart","ISDateStop",outcome_full)

##df.med is medication data frame of interest eg IS or FiveASA
##type is datapoint recording type of medication (eg 6MP or AZA or MTX)
##"values" is a vector of numeric codes of medications to include in the analysis eg c(1,2,6) which are the thiopurines (see isstring_word)
##"datestart" is date vector of the start date of the medication eg. "ISDateStart"
##"datestop" is the date vector of stop date of the medication eg. "ISDateStop"
##df.outcome is the dataframe of outcomes - usually df.outcome is "outcome_full". This dataframe needs to have the fields "start", "Date","id"

vector<-rep(0,length(df.outcome$id))
vector2<-rep(NA,length(df.outcome$id))
test<-df.med[,type]%in%values
df.med2<-subset(df.med,test)
for(i in 1:length(df.outcome$id)){
  id<-df.outcome$id[i]
  t.test<-df.med$id==id
  df.med3<-subset(df.med2,t.test)
  if(length(df.med3[,1])>0){
    df.med3$mark<-NA
    df.med3$mark<-ifelse(as.Date(df.med3[,datestart])>as.Date(df.outcome$Date)[i] | as.Date(df.med3[,datestop])<as.Date(df.outcome$start)[i],0,1)
```

```
    df.med3$start<-ifelse(as.Date(df.med3[,datestart])<as.Date(df.outcome$start)[
    i],df.outcome$start[i],df.med3[,datestart])
    df.med3$stop<-ifelse(as.Date(df.med3[,datestop])>as.Date(df.outcome$Date)[i],
    df.outcome$Date[i],df.med3[,datestop])
    df.med3<-subset(df.med3,df.med3$mark==1)
    num<-sum(as.numeric(as.Date(df.med3$stop)-as.Date(df.med3$start)),na.rm=TRUE)
    denom<-as.numeric(as.Date(df.outcome$Date)[i]-as.Date(df.outcome$start)[i])
    if(!is.na(num/denom)){
      vector[i]<-num/denom
      }
    print(i)
    }
  denom<-as.numeric(as.Date(df.outcome$Date)[i]-as.Date(df.outcome$start)[i])
  vector2[i]<-denom
  }
vector3<-cbind(vector,vector2)
return(vector3)
}
```

**Listing 10**

Medication exposure

## .1.5. Laboratory variable processing

These functions are used to calculate a representative number for a laboratory variable in the observation period preceding an outcome of interest. The code also provides the duration of the observation period and the number of tests used to calculate the number.

```
fun_AUC_vector<-function(df,lab,lab_df,Area_function,exclude_surg){
##function to create a vector of modified AUC values during appropriate time
    period for each outcome.
#df is dataframe being analysed eg. < outcome_full >, or <outcome_surgery >.
##lab is laboratory variable of interest eg "PLT"
##lab_df is name of dataframe in which variable sits eg. allHGB
##Area function is either AUC1 or AUC2. AUC1 weighs towards lowest value (for
    variables which are raised if patients are unwell/disease is active). AUC1
    weighs towards highest value, for variables which drop as patients become
    unwell.
##exclude_surg; either true or false: if true then exclude all lab tests within "
    surgery_cut" of an operation.
if(exclude_surg==TRUE){
```

```
    lab_df<-fun_exclude_near_surgery(lab_df,surgery_cut)
    lab_df<-subset(lab_df,!lab_df$near_surgery)
    }
lab_obs<-paste(lab,"_obs",sep="")
lab_duration<-paste(lab,"_duration",sep="")
df[,lab]<-NA
df[,lab_obs]<-NA
df[,lab_duration]<-NA
len<-length(df[,1])
for(i in 1:len){
id<-df$id[i]
temp<-lab_df[lab_df$RegID==id,c("collected",lab)]
t.start<-ifelse(cd$DateDiag1[cd$id==id]==df$start[i],as.character(as.Date(df$
    start[i])+observation_cut_diag),df$start[i])
include<-(as.Date(temp[,"collected"])<(as.Date(df$Date[i])-observation_cut_
    outcome) & (as.Date(temp[,"collected"])>as.Date(t.start)) & (as.Date(temp[,"
    collected"])<as.Date(t.start)+observation_cut_end))
temp<-temp[include,]
if(length(temp[,1])>1 & !is.na(t.start) & !is.na(df$Date[i])){
temp<-temp[order(temp$collected),]
med<-Area_function(lab,"collected",temp)
duration<-as.numeric(max(as.Date(temp$collected),na.rm=TRUE)-min(as.Date(temp$
    collected),na.rm=TRUE))/365
df[i,lab]<-med
df[i,lab_obs]<-length(temp[,1])
df[i,lab_duration]<-duration
}
}
return(df)
}
```

**Listing 11**

Master function to calculate area under curve of a laboratory variable

```
AUC<-function(a,b,c){
##a is data of interest eg Platelet count "PLT"
##b is date vector eg "collected"
##c is dataframe
##function calculates a rolling 'mean' of data, however instead of mean a value
    val<-x+(y-x)/5 represents a middle value (x is the lower of the two values)
test<-!is.na(c[,a]) & !is.na(c[,b])
temp<-c[test,]
l<-length(temp[,a])
if(l>1){
tmp<-rep(NA,(l-1))
for (i in 2:l){
low<-min(as.numeric(temp[(i-1):i,a]))
high<-max(as.numeric(temp[(i-1):i,a]))
```

```
val<-low+(high-low)/5
tmp[i-1]<-val
}
AUC <- sum(diff(as.numeric(as.Date(temp[,b])))*tmp,na.rm=TRUE)
AUC2<-AUC/as.numeric((max(as.numeric(as.Date(temp[,b])))-min(as.numeric(as.Date(
    temp[,b]))))))
}else{
AUC2<-NA
}
return(AUC2)
}
```

**Listing 12**

Area under curve of a laboratory variable: For variables that tend to rise with inflammation

```
AUC2<-function(a,b,c){
##a is data of interest eg Platelet count "PLT"
##b is date vector eg "collected"
##c is dataframe
##function calculates a rolling 'mean' of data, however instead of mean a value
    val<-x+(y-x)/5 represents a middle value (x is the lower of the two values)
test<-!is.na(c[,a]) & !is.na(c[,b])
temp<-c[test,]
l<-length(temp[,a])
if(l>1){
tmp<-rep(NA,(l-1))
for (i in 2:l){
low<-min(as.numeric(temp[(i-1):i,a]))
high<-max(as.numeric(temp[(i-1):i,a]))
val<-low+4*(high-low)/5
tmp[i-1]<-val
}
AUC <- sum(diff(as.numeric(as.Date(temp[,b])))*tmp,na.rm=TRUE)
AUC2<-AUC/as.numeric((max(as.numeric(as.Date(temp[,b])))-min(as.numeric(as.Date(
    temp[,b]))))))
}else{
AUC2<-NA
}
return(AUC2)
}
```

**Listing 13**

Area under curve of a laboratory variable: For variables that tend to fall with inflammation

```
fun_exclude_near_surgery<-function(a,cutoff_near_surgery){
```

```
##this function excludes lab results which occur near a surgery for each patient
    (those with a TRUE in column 2$near_surgery are to be excluded).
##a is dataframe eg allPLT
##cutoff_near_surgery is the time period either side of a surgical event in days
    (eg 30) in which lab data is not used.
##this function uses the dataframe < Surgery > which includes all surgical
    procedures regardless of what the indication or surgery type.
a$near_surgery<-rep(FALSE, length(a[,1]))
limit<-duplicated(Surgery$id)
t.Surgery<-subset(Surgery,!limit)
for (i in 1:length(t.Surgery[,1])){
  id<-t.Surgery[i,"id"]
  test<-Surgery[,"id"]==id
  t.Surgery2<-subset(Surgery,test)
  test2<-a$RegID==id
  t.a<-subset(a,test2)

  if(length(t.a[,1])>0){
    mat<-matrix(data = NA, nrow = length(t.Surgery2[,1]), ncol = length(t.a[,1]),
     byrow = FALSE, dimnames = NULL)
    for(j in 1:length(t.Surgery2[,1])){
      mat[j,]<-abs(as.Date(t.a$collected)-as.Date(t.Surgery2[j,"SurgeryDate"]))<
    cutoff_near_surgery
      }
    out<-rep(NA,length(t.a[,1]))
    for(k in 1:length(t.a[,1])){
      out[k]<-TRUE %in% mat[,k]
      }
    a$near_surgery[test2]<-out
    }
  }
return(a)
}
```

**Listing 14**

Exclusion of laboratory data around time of surgery

## .1.6. Conversion of continuous to categorical variables

The following code automates a process to identify the value which is best used as a cutoff to convert continuous to categorical variables. This value is selected comparing the log rank of association by univariate cox regression between a categorical

variable and the outcome of interest, using a varying cutoff. It also generates a graphical representation of the calculation in *.jpg* format.

```
fun_cutoff_graph<-function(low,high,gap,lab,lablong1,lablong2){
###function to dertmine optimal cutoff for converting continuous to categorical variable
##low is lower limit of cutoff to be examined
##high is higer limit of cutoff to be examined
##gap is the jump in value each iteration (eg < 1 > gives 13,14,15, < 0.1 > gives 13.1, 13.2, 13.3)
#lab is the name of the datapoint eg "ALB"
#lablong1 is the name of the datapoint for naming the file, and for the title of the graph eg. "Albumin"
#lablong2 is appropriate descriptor of < lablong > eg "Count", or "Level"
test<-seq(low,high,by=gap)
temp<-data.frame(lab_value=character(),
                 LogRank=character(),
                 stringsAsFactors=FALSE)
for (i in 1:length(test)){
coxfit<-NA
outcome_full$temp<-ifelse(outcome_full[,lab]<test[i],1,0)
coxfit<-coxph(Surv(outcome_full$surv,outcome_full$event)~ temp, data=outcome_full)
temp2<-c(test[i],coxfit$score)
temp<-rbind(temp,temp2)
}
colnames(temp)<-c(lab,"LogRank")
temp$running<-NA
for(i in 3:(length(temp[,1])-2)){
temp$running[i]<-sum(temp$LogRank[(i-2):(i+2)])/5
}
cutoff<-temp[which.max(temp$running),lab]
colnames(temp)<-c(lab,"LogRank")
grey_jpg4by3(paste(lablong1,"cutoff",sep="_"))
plot(temp[,1:2],col='blue',type='l',main=paste("Continous to Categorical: \nOptimal cutoff for ",lablong1,lablong2,sep=" "),xlab=paste(lablong1,lablong2,sep=" "))
dev.off()
return(cutoff)
}
```

**Listing 15**

Calculation of optimal cutoff for conversion of continuous to categorical variables

```
fun_sig_cox_univariate2<-function(coxfit){
```

```
##function to display significance of log.rank test for univariate analysis)
##coxfit represents the output of cox regression.
t.temp<-summary(coxfit)
t.out<-unlist(c(unlist(t.temp)[c("n","nevent","coefficients2","logtest.pvalue")
    ],(length(unlist(t.temp))-28)))
t.out2<-c(t.out[1],t.out[c(2,3,4)])
return(t.out2)
}
```

**Listing 16**

Display of univarite cox regression analysis

```
fun_sig_cox_univariate_multilevel<-function(coxfit){
##function to display significance of log.rank test for univariate analysis)
##coxfit represents the output of cox regression.
t.temp<-summary(coxfit)
t.n<-as.numeric(unlist(t.temp)["logtest.df"])+1
t.n.vec<-c(seq(t.n,(t.n+(t.n-2)),by=1),seq(3*t.n,(3*t.n+(t.n-2)),by=1))
t.coefficient<-paste("coefficients",t.n.vec,sep="")
t.out<-matrix(rep(NA,(t.n-1)*5),nrow=(t.n-1),ncol=5)
for (i in 1:t.n-1){
t.out[i,]<-unlist(c(table(grepl("na.action",names(unlist(t.temp))))[2],unlist(t.
    temp)[c("n","nevent",t.coefficient[c(i,(i+(t.n-1)))])]))
}
t.out<-as.data.frame(t.out)
t.out$number<-t.out[,1]+t.out[,2]
t.out<-t.out[,c(6,2:5)]
return(t.out)
}
```

**Listing 17**

Display of univarite cox regression analysis, for regression using mulitlevel categorical variables

## .2. Montreal disease classification

The following functions define Montreal Disease Classification at a given time-point in the disease course - For eg. at diagnosis, or at time of first surgery, or last follow-up.

```
fun_montreal_location<-function(a,b){
##function to determine extent
```

```
##dataframe is dataframe in which vectors reside
##a = ileal extent vector eg "extent.ileal.dx"
##b = colonic extent vector
temp1<-ifelse(a>0,
ifelse(b>0,"L3","L1"),
ifelse(b>0,"L2","L0"))
return(temp1)
}
```

**Listing 18**

Daughter function to calculate disease location as defined by the Montreal Classification System

```
##montreal classification function
montreal_location<-function(Datefield,locationCutoff){
##locationCutoff is the time in days after the date at which location is being determined in which it is permissible to use location data (eg a colonoscopy 6 months after diagnosis).
##suffix is the suffix to add to all colnames for this montreal classification - either ".dx" ".LFU" or something else depending on timepoint.

##ileal extent at date.
t.rad<-fun_extent(RadAbdo2,"RadAbdoDate","RadAbdoExtent",grepsmallbowel,cd,Datefield,locationCutoff)
t.colo<-fun_extent(Colo,"ColoDate","ColoExtent",grepsmallbowel,cd,Datefield,locationCutoff)
t.colo.hist<-fun_extent_double(Colo,"ColoDate","ColoHistoMicroExtent",grepsmallbowel,"ColoHistoMicroCoded",grephisto,cd,Datefield,locationCutoff)
t.surg<-fun_extent_double(Surgery2,"SurgeryDate","SurgHistoMicroExtent",grepsmallbowel,"SurgeryHistoMicroCoded",grephisto,cd,Datefield,locationCutoff)
location.ileal<-fun_extentMaster(t.rad,t.colo,t.surg)

##colonic extent at date.
t.rad<-fun_extent(RadAbdo2,"RadAbdoDate","RadAbdoExtent",grepcolo,cd,Datefield,locationCutoff)
t.colo<-fun_extent(Colo,"ColoDate","ColoExtent",grepcolo,cd,Datefield,locationCutoff)
t.colo.hist<-fun_extent_double(Colo,"ColoDate","ColoHistoMicroExtent",grepcolo,"ColoHistoMicroCoded",grephisto,cd,Datefield,locationCutoff)
t.surg<-fun_extent_double(Surgery2,"SurgeryDate","SurgHistoMicroExtent",grepcolo,"SurgeryHistoMicroCoded",grephisto,cd,Datefield,locationCutoff)
location.colo<-fun_extentMaster(t.rad,t.colo,t.surg)
location.montreal<-fun_montreal_location(location.ileal,location.colo)

print(paste("Location Data from longitudinal database at < datefield > timepoint - L0 means no evidence of bowel involvement on imaging/endoscopy/surgery",sep=" "))
```

```
print(table(location.montreal))

temp<-location.montreal
for(i in 1:length(cd[,1])){
if(location.montreal[i]=="L0"&cd$id[i]%in%pri$id){temp[i]<-pri[pri$id==cd$id[i],"
    LocationLFU"]}
}
temp2<-ifelse(is.na(temp),"missing",
ifelse(temp=="1","L1",
ifelse(temp=="2","L2",
ifelse(temp=="3","L3",
ifelse(temp%in%c("L0","4"),"missing",temp)))))
return(temp2)
}
```

**Listing 19**

Master function to calculate disease location as defined by the Montreal Classification System

```
fun_montreal_behaviour<-function(dataframe,a,b){
##function to determine behaviour
##dataframe is dataframe in which vectors reside
##a = perforation vector
##b = stenosis vector
temp1<-ifelse(dataframe[,a]>0,
ifelse(dataframe[,b]>0,"B32","B3"),
ifelse(dataframe[,b]>0,"B2","B1"))
return(temp1)
}
```

**Listing 20**

Daughter function to calculate disease behaviour as either inflammatory, stricturing or penetrating, or penetrating and stricturing.

```
##montreal classification function
montreal_behaviour<-function(Datefield,Cutoff){
##datefield is the time when analysis is to be made eg "LstSeen" or "DateDiag"
##Cutoff is the time in days after the date at which location is being determined
     in which it is permissible to use location data (eg a colonoscopy 6 months
    after diagnosis).
##suffix is the suffix to add to all colnames for this montreal classification -
    either ".dx" ".LFU" or something else depending on timepoint.

##penetrating disease at lfu. (abcess, fistula)
t.rad<-fun_extent(RadAbdo2,"RadAbdoDate","RadAbdoFist",grepbowel,cd,Datefield,
    Cutoff)
t.rad2<-fun_extent(RadAbdo2,"RadAbdoDate","RadAbdoOther",grepabcessRad,cd,
    Datefield,Cutoff)
```

```
t.colo<-fun_extent(Colo,"ColoDate","ColoFist",grepbowel,cd,Datefield,Cutoff)
t.surg1<-fun_extent(Surgery2,"SurgeryDate","SurgeryFist",grepbowel,cd,Datefield,
    Cutoff)
t.surg2<-fun_extent(Surgery2,"SurgeryDate","SurgeryOther",grepabcessSurg,cd,
    Datefield,Cutoff)
t.surg3<-fun_extent(Surgery2,"SurgeryDate","SurgeryPerf",grepabcessSurg,cd,
    Datefield,Cutoff)
t.pen<-fun_extentMaster(t.rad,t.rad2,t.colo,t.surg1,t.surg2,t.surg3)
t.pen<-fun_binary(t.pen)

##stenosis at lfu.
t.rad<-fun_extent_double(RadAbdo2,"RadAbdoDate","RadAbdoStenosis",grepbowel,"
    RadAbdoStenosisDilation",grepDilation,cd,Datefield,Cutoff)
t.colo<-fun_extent(Colo,"ColoDate","ColoStenosis",grepbowel,cd,Datefield,Cutoff)
t.surg<-fun_extent(Surgery2,"SurgeryDate","SurgeryStenosis",grepbowel,cd,
    Datefield,Cutoff)
t.sten<-fun_extentMaster(t.rad,t.colo,t.surg)
t.sten<-fun_binary(t.sten)

##montreal at lfu.
t.mon.behaviour<-fun_montreal_behaviour_vector(t.pen,t.sten)
return(t.mon.behaviour)
}
```

**Listing 21**

Master function to calculate disease behaviour as either inflammatory, stricturing or penetrating, or penetrating and stricturing. This is similar to the Montreal Classification System

```
##montreal classification function
montreal_perianal_modifier<-function(Datefield,locationCutoff){
##locationCutoff is the time in days after the date at which location is being
    determined in which it is permissible to use location data (eg a colonoscopy
    6 months after diagnosis).
##suffix is the suffix to add to all colnames for this montreal classification -
    either ".dx" ".LFU" or something else depending on timepoint.
##perianal disease at date
t.PAclin<-fun_extent(PAclinical,"PAClinicalDate","PAClinicalFistula",grepfistula,
    cd,Datefield,locationCutoff)
t.PArad<-fun_extent(RadPA,"RadPADate","RadPAFistula",grepfistula,cd,Datefield,
    locationCutoff)
t.PAsurg.fist<-fun_extent(PASurgery,"PASurgeryDate","PASurgeryFistula",
    grepfistula,cd,Datefield,locationCutoff)
#t.PAsurg.abcess<-fun_extent(PASurgery,"PASurgeryDate","PASurgeryAbcess",
    grepabcess,cd,Datefield,locationCutoff)
t.PA<-fun_extentMaster(t.PAclin,t.PArad,t.PAsurg.fist)
t.PA<-ifelse(t.PA>0,1,0)
```

```
return(t.PA)
}
```

**Listing 22**

Master function to calculate perianal disease modifier as defined by the Montreal Classification System

```
##montreal classification function
montreal_upperGI_modifier<-function(Datefield,locationCutoff){
##locationCutoff is the time in days after the date at which location is being determined in which it is permissible to use location data (eg a colonoscopy 6 months after diagnosis).
##suffix is the suffix to add to all colnames for this montreal classification - either ".dx" ".LFU" or something else depending on timepoint.
##perianal disease at date
t.upper.hist<-fun_extent_double(Gas,"GastroDate","GastroHistoMicroExtent",grepupper,"GastroHistoMicroCoded",grephisto.upper,cd,Datefield,locationCutoff)
t.temp<-fun_extentMaster(t.upper.hist)
t.temp<-ifelse(t.temp>0,1,0)
return(t.temp)
}
```

**Listing 23**

Master function to calculate upper GI disease modifier as defined by the Montreal Classification System

## .3. Fistula, stenosis or perforation occurence

The definition of occurence of intestinal fistula, stenosis or perforation (outcome) is defined here. This definition is made based on data from colonoscopy, gastroscopy, radiological procedures, surgery, histology from surgical specimens, and clinical examination events. There are multiple observations by multiple modalities of outcomes (eg. on CT, colonoscopy, at surgery). This code makes an assessment as to whether observed outcomes are repeat observations of previously observed outcomes, or if they are observations of a new outcome.

## .3.1. Time cutoffs

These cutoffs are the time periods used to distinguish whether an observed outcome is a repeat observation of a previously observed outcome, or is a new outcome.

```
1
2 ##cutoff_passive_independence: cutoff in days for measuring whether an outcome
      event is new, or if it is observation of a previously observed outcome event.
3 cutoff_passive_independence<-730
4
5 ##cutoff_surgical_independence: cutoff in days for measuring whether an event is
      new, or if it is observation of a previously observed outcome which has not
      been resolved by intervening surgery. This cutoff may be shorter than passive
       independence cutoff as surgery is usually associated with resolution of
      outcome event.
6 cutoff_surgical_independence<-730
7
8 ##passive resolution_cutoff: cutoff in days for measuring whether an event has
      resolved. This time must pass without observation of the outcome for it to be
       considered passively resolved. If the outcome is observed again (one or more
       times) then 'passive_resolution_cutoff' must then pass from the latest date
      it was observed, for the outcome to be considered resolved.
9 passive_resolution_cutoff<-cutoff_passive_independence
10
11 ##surgical resolution_cutoff: cutoff in days for measuring whether an event has
      resolved. This time must pass without observation of the outcome, after
      surgery resecting the affected bowel, for the outcome to be considered
      surgically resolved. If the outcome is observed again (one or more times)
      then 'passive_resolution_cutoff' must pass from the latest date it was
      observed, for the outcome to be considered resolved.
12 surgical_resolution_cutoff1<-cutoff_surgical_independence
```

**Listing 24**

Cutoff definitions used to define indepedence of observed outcomes

## .3.2. Master code to process definition

The output of this code is a table with a line for each outcome. There may be multiple lines per patient. To allow regression analysis a line is added for patients who have not suffered an outcome at the end of the study period. This added

line has *"no outcome, censored"* as the identified outcome and will be used as a censored event in analysis.

```
##now create outcome table
##create outcome dataframes for individual endpoints (possibly multiple per
    patient over disease course)
obs<-"Fist$"
sub_code(path_in,"outcome")
outcome.fistula<-tt.outcome2
##remove temp files (those starting with 't.' and 'tt.')
rm(list=ls()[grepl("^t\\.",ls())])
rm(list=ls()[grepl("^tt\\.",ls())])

obs<-"Stenosis$"
sub_code(path_in,"outcome")
outcome.stenosis<-tt.outcome2
##remove temp files (those starting with 't.' and 'tt.')
rm(list=ls()[grepl("^t\\.",ls())])
rm(list=ls()[grepl("^tt\\.",ls())])

obs<-"Other$|Perf$"
sub_code(path_in,"outcome_abcess_perf")
outcome.perforation<-tt.outcome2
##remove temp files (those starting with 't.' and 'tt.')
rm(list=ls()[grepl("^t\\.",ls())])
rm(list=ls()[grepl("^tt\\.",ls())])

##create full outcome dataframe
sub_code(path_in,"outcome_full")

##remove temp files (those starting with 't.' and 'tt.')
rm(list=ls()[grepl("^t\\.",ls())])
```

**Listing 25**

Master code to calculate when independent fistulae, perforations or stenoses occur. Those that are observed within a certain time of a previously observed event are considered a repeat observation of the same event and treated as such.

## .3.3. Independent fistula or stenosis occurrence

This code outputs a table with one line per row for each independent event (eg. Fistula). Only one type of event is analyzed. There may be multiple lines per patient. It can calculate "Fistula" and "Stenosis" but not "Perforation". "Perforation" is coded by "outcome_abcess_perf". Perforation, fistula and stenosis events are then combined into one table using the code "outcome_full".

```
## 'out' is the boolean vector which describes whether outcome has been met in observed procedure
##modality is the modality of the observing procedure

#create vector of column names of three observation dataframes
t.coloNames<-colnames(Colo)
t.radNames<-colnames(RadAbdo2)
t.surgNames<-colnames(Surgery2)
t.allNames<-c(t.coloNames,t.radNames,t.surgNames)

tt.out<-t.allNames[grepl(obs,t.allNames)]
tt.date<-t.allNames[grepl("Date",t.allNames)]
##'attribute' define outcome attribute (for stenosis this is radiological upstream dilatation)
attribute<-t.allNames[grepl("StenosisD",t.allNames)]
attribute2<-t.allNames[grepl("SurgeryL",t.allNames)]

##define colnames of outvector
##LocString is location of outcome eg. '6,7'   (terminal ileum and caecum).
t.outNames<-c("id","Date","LocString","out","modality")

##clean outcome field of interest (no 1's)
#note will need to review fields in this coding at a later date.
##change 1 to 0 if it exists on it's own in stenosis strings (likely coding error will need to be reviewed at a later date).
RadAbdo2[,t.radNames%in%tt.out]<-ifelse(RadAbdo2[,t.radNames%in%tt.out]==1,0,RadAbdo2[,t.radNames%in%tt.out])
Colo[,t.coloNames%in%tt.out]<-ifelse(Colo[,t.coloNames%in%tt.out]==1,0,Colo[,t.coloNames%in%tt.out])
Surgery2[,t.surgNames%in%tt.out]<-ifelse(Surgery2[,t.surgNames%in%tt.out]==1,0,Surgery2[,t.surgNames%in%tt.out])

#########################
##(merge all modalities)
#########################
#RadAbdo2
```

```
RadAbdo2$out<-ifelse(!(RadAbdo2[,t.radNames%in%tt.out]==0 | is.na(RadAbdo2[,t.
    radNames%in%tt.out])),1,0)
RadAbdo2$modality<-"radiology"
#Colo
Colo$out<-ifelse(!(Colo[,t.coloNames%in%tt.out]==0 | is.na(Colo[,t.coloNames%in%
    tt.out]) | grepl("[a-zA-Z]",Colo[,t.coloNames%in%tt.out])),1,0)
Colo$modality<-"colonoscopy"
#Surgery2
Surgery2$out<-ifelse(!(Surgery2[,t.surgNames%in%tt.out]==0 | is.na(Surgery2[,t.
    surgNames%in%tt.out]) | grepl("[a-zA-Z]",Surgery2[,t.surgNames%in%tt.out]))
    ,1,0)
Surgery2$modality<-"surgery"

##########################
##select columns of interest only and give them unifying names
##########################
#define function
unify<-function(f.string,f.names,f.table.original){
##f.string -> string on which to search attributes columns to identify which
    table they are in.
##f.names -> string of columnnames of the table to be modified eg "t.surgNames"
##f.table -> name of temporary table to be created eg t.Surgery2
##f.table2 -> name of original table eg Surgery2
t.test<-!grepl(f.string,attribute)
t.test2<-!grepl(f.string,attribute2)
t.insert<-c("id",f.names[f.names%in%tt.date],f.names[f.names%in%tt.out],"out","
    modality")
if(t.test){
  if(t.test2){
    f.table<-f.table.original[,t.insert]
    f.table$filler<-NA
    f.table$filler2<-NA
    }else{
    f.table<-f.table.original[,t.insert]
    f.table$filler<-NA
    f.table$filler2<-f.table.original[,attribute2]
  }}else{
  if(t.test2){
    t.insert<-c(t.insert,attribute)
    f.table<-f.table.original[,t.insert]
    f.table$filler2<-NA
    }else{
    t.insert<-c(t.insert,attribute,attribute2)
    f.table<-f.table.original[,t.insert]
    }
  }
return(f.table)
}
```

```
73
74 t.Surgery2<-unify("Surg",t.surgNames,Surgery2)
75 t.RadAbdo2<-unify("Rad",t.radNames,RadAbdo2)
76 t.Colo<-unify("Colo",t.coloNames,Colo)
77
78 t.outNames2<-c(t.outNames,"attribute","attribute2")
79 colnames(t.Colo)<-t.outNames2
80 colnames(t.Surgery2)<-t.outNames2
81 colnames(t.RadAbdo2)<-t.outNames2
82 tt.outcome<-rbind(t.Colo,t.Surgery2,t.RadAbdo2)
83 tt.outcome<-tt.outcome[!is.na(tt.outcome$Date),]
84
85 ##########################
86 ##if outcome is STENOSIS:
87 ##########################
88 # #1    Seperate into multiple rows where more than one stenosis exists.
89 # #2    Additionally remove anal stenosis from this analysis (at a later point
      these could be moved to PAclinical field).
90 # #3    Recode as no stenosis if 'attribute' (stenosis_dilation) <2.5cm
91 # #4    Remove lines where a second stenosis has been observed on the same
      examination in the same location.
92 #1
93 if(TRUE%in%grepl("Stenosis",tt.out)){
94 t.mult<-grepl(",",tt.outcome$LocString)
95   if(TRUE%in%grepl("TRUE",t.mult)){
96   t.temp<-tt.outcome[t.mult,]
97   for(i in 1:length(t.temp[,1])){
98     t.temp2<-strsplit(t.temp$LocString[i],",")
99     t.temp3<-strsplit(t.temp$attribute[i],",")
100    t.len<-length(t.temp2[[1]])
101    t.temp4<-t.temp[rep(i, each=t.len),]
102    t.temp4[,"LocString"]<-t.temp2
103    t.temp4[,"attribute"]<-t.temp3
104    t.temp<-rbind(t.temp,t.temp4)
105    }
106  t.temp<-t.temp[!grepl(",",t.temp$LocString),]
107  tt.outcome<-rbind(tt.outcome,t.temp)
108  }
109 #2
110 tt.outcome<-tt.outcome[!(grepl(",",tt.outcome$LocString)|tt.outcome$LocString
      ==12|is.na(tt.outcome$LocString)),]
111 #3
112 tt.outcome$out<-ifelse(tt.outcome$modality=="radiology" & (is.na(tt.outcome$
      attribute) | tt.outcome$attribute<2.5),0,tt.outcome$out)
113 #4
114 tt.outcome<-tt.outcome[!duplicated(paste(tt.outcome$Date,tt.outcome$id,tt.outcome
      $LocString)),]
115 }
```

```
116
117 ##########################
118 ##if outcome is FISTULA:
119 ##########################
120 ##remove attribute (fistulae do not have an attribute)
121 tt.outcome$attribute<-NA
122
123 ##remove temp files (those starting with 't.')
124 rm(list=ls()[grepl("^t\\.",ls())])
125
126 ##########################
127 ##identify outcomes which qualify as independent outcome events, observed on
        radiology, colonoscopy or surgery.
128 ##########################
129
130 # An old outcome is one that has been identified previously on radiology,
        colonoscopy or at surgery, prior to time period defined by 'cutoff_passive_
        independence' and 'cutoff_surgical_independence'.
131 #   A recent outcome is one identified within time period before the outcome
        being assessed (as defined by 'cutoff_passive_independence' and 'cutoff_
        surgical_independence').
132 # 'cutoff_passive_independence' is used to assess whether most prior outcomes are
         'old' or 'recent'.
133 # If a prior outcome is surgical resection of affected segment,'cutoff_surgical_
        independence' is used to assess whether it is 'old' or 'recent'.(this cutoff
        is shorter, acknowledging that surgery usually resolves stenosis/fistulae).
134 # If no recent outcome exists then the outcome being assessed is considered
        independent.
135
136 tt.outcome2<-data.frame(id=character(),
137                         Date=character(),
138                         LocString=character(),
139     out=character(),
140     outcome=character(),
141     attribute=character(),
142     modality=character(),
143                         stringsAsFactors=FALSE)
144
145 for (i in 1:length(cd[,1])){
146 id<-cd$id[i]
147 t.temp<-tt.outcome[tt.outcome$id==id,]
148 t.temp<-t.temp[order(t.temp$Date),]
149 if(length(t.temp[,1])>0){
150 t.temp$outcome<-NA
151 t.temp[1,"outcome"]<-t.temp$out[1]
152 if(length(t.temp[,1])>1){
153 for (j in 2:length(t.temp[,1])){
```

```
##in the 'cutoff_surgical_independence' period before this test, was an outcome observed? (whether or not there has been intervening surgery).
t.compareAll<-subset(t.temp,as.Date(t.temp$Date[j])-as.Date(t.temp$Date)<cutoff_surgical_independence & as.Date(t.temp$Date[j])-as.Date(t.temp$Date) > 0 & t.temp$out==1)
##in the period between 'cutoff_passive_independence' and 'cutoff_surgery_independence' before this test, was an outcome which was not "surgery resecting involved segment" observed? (If it is a surgical resection in this time period it is considered 'old', otherwise it is considered recent).
t.comparePassive<-subset(t.temp,as.Date(t.temp$Date[j])-as.Date(t.temp$Date)<cutoff_passive_independence & as.Date(t.temp$Date[j])-as.Date(t.temp$Date) >= cutoff_surgical_independence & t.temp$out==1 & (!t.temp$modality=="surgery" | grepl(t.temp$LocString[j],t.temp$attribute2)))
##code only independent outcomes as '1' in
t.temp$outcome[j]<-ifelse(t.temp$out[j]==0 | length(t.compareAll[,1])>0 | length(t.comparePassive[,1])>0,0,1)
}}}
t.temp2<-subset(t.temp,t.temp$outcome==1)
tt.outcome2<-rbind(tt.outcome2,t.temp2)
}
tt.outcome2$outcomeID<-c(1:length(tt.outcome2[,1]))

##remove temp files (those starting with 't.')
rm(list=ls()[grepl("^t\\.",ls())])

#########################
#define resolution
#########################
##definition of resolution of outcome:
##subsequent surgery resecting affected segment of bowel within 'surgical_resolution_cutoff'; or
##Once "passive_resolution_cutoff' time has passed without recurrent observation of stenosis.

##add colnames needed for resolution data.
t.resolutionNames<-c("DateResolution","resolution","SurgeryLocResolution")
for(name in t.resolutionNames){
tt.outcome2[,name]<-NA
}

for (i in tt.outcome2$outcomeID){
  ##reset resolution vector to NA
  t.resolution<-NA
  ##id of patient for this outcome event
  t.id<-tt.outcome2$id[tt.outcome2$outcomeID==i]
  ##isolate outcome event
  t.out<-tt.outcome2[tt.outcome2$outcomeID==i,]
  t.temp<-tt.outcome[tt.outcome$id==t.id,]
```

```
190
191 #determine date of resolution (passive or surgical)
192 ##resMarker: 1 means that this investigation demonstrates that the outcome being
      observed has not yet resolved: 0 means that it has resolved (over    <
      passive_resolution_cutoff >    has passed without observation of outcome).
193   t.passRes<-t.temp[as.numeric(as.Date(t.temp$Date)-as.Date(t.out$Date)) > 0 & t.
      temp$out==1 ,]
194   t.passRes<-rbind(t.out[,colnames(t.passRes)],t.passRes)
195   t.passRes<-t.passRes[order(t.passRes$Date),]
196   t.len<-length(t.passRes[,1])
197   ##if surgery was affected segment removed?
198   t.rightLoc<-rep(NA,length(t.passRes[,1]))
199   t.grep<-(grepstringvector(t.passRes$attribute2))
200   for ( j in 1:length(t.passRes[,1])){
201     t.rightLoc[j]<-grepl(t.grep[j],t.out$LocString)
202     }
203   t.passRes$resMarker<-NA
204   t.passRes$resMarker[1]<-1
205   if (length(t.passRes[,1])>1){
206     for (j in 2:length(t.passRes[,1])){
207       ##if preceding examintion resection of affected segment, use    < surgical_
      resolution_cutoff1 > otherwise use    < passive_resolution_cutoff >
208       if(t.passRes$modality[j-1]=="surgery" & t.rightLoc[j-1]==TRUE){
209         t.cutoff<-surgical_resolution_cutoff1
210         }else{
211         t.cutoff<-passive_resolution_cutoff
212         }
213       ##if t.cutoff hasn't passed, mark resMarker < 1 >.
214       if(as.Date(t.passRes$Date[j])-as.Date(t.passRes$Date[j-1]) <t.cutoff & t.
      passRes$resMarker[j-1]>0){
215         t.passRes$resMarker[j]<-1
216         }else{t.passRes$resMarker[j]<-0
217         }
218       }
219     ##mark resMarker2 as < 1 > for the last investigation that observes the
      unresolved outcome.
220     t.passRes$resMarker2<-NA
221     for (j in 2:length(t.passRes[,1])){
222       if(t.passRes$resMarker[j-1]==1 & t.passRes$resMarker[j]==0){
223         t.passRes$resMarker2[j-1]<-1
224       }
225     }
226   ##for the last observation, mark resMarker2 as < 1 > if patient has been
      observed for < t.cutoff > time and outcome has not been observed again.
227   t.cutoff<-NA
228   t.end<-cd[cd$id==t.id,"LstSeen"]
229   if(is.na(t.end)){t.end<-as.character(Sys.Date())}
```

```
230     ##if last examintion is resection of affected segment, use    < surgical_
        resolution_cutoff1 > otherwise use   < passive_resolution_cutoff >
231     if(t.passRes$modality[t.len]=="surgery" & t.rightLoc[t.len]==TRUE){
232       t.cutoff<-surgical_resolution_cutoff1
233       }else{
234       t.cutoff<-passive_resolution_cutoff
235       }
236     ##mark resMarker2 as < 1 > for the last investigation if patient has been
        observed for < t.cutoff > time and outcome has not been observed again.
237     if(t.passRes$resMarker[t.len]==1&as.Date(t.end)-as.Date(t.passRes$Date[t.len
        ])>t.cutoff){
238       t.passRes$resMarker2[t.len]<-1
239       }
240     ##select resolution observation ( where resMarker2 = 1 )
241     t.resolution<-subset(t.passRes,!is.na(t.passRes$resMarker2)&t.passRes$
        resMarker2==1)
242     ##assign date of resolution (after appropriate resolution cutoff period has
        passed).
243     if(length(t.resolution[,1])>0){
244       t.resolution<-t.resolution[,c("Date","modality","attribute2")]
245       t.resolution$Date<-ifelse(t.resolution$modality=="surgery",as.character(as.
        Date(t.resolution$Date)+surgical_resolution_cutoff1),as.character(as.Date(t.
        resolution$Date)+passive_resolution_cutoff))
246       }else{
247       t.resolution<-c(t.end,"no_resolution",NA)
248       }
249     }
250 ##above nested code assigns resolution where more than one observation exists
        after the initial observation. For those with only the initial observation,
        code below assigns resolution dependant on whether < resolution cutoff > has
        passed before the end of the observation period.
251   if(!TRUE%in%grepl("[a-z0-9]",t.resolution)){
252     t.end<-cd[cd$id==t.id,"LstSeen"]
253     if(is.na(t.end)){t.end<-as.character(Sys.Date())}
254     if(t.passRes$modality[1]=="surgery"){
255       t.cutoff<-surgical_resolution_cutoff1
256       }else{
257       t.cutoff<-passive_resolution_cutoff
258       }
259     if(as.Date(t.end)-as.Date(t.passRes$Date)<t.cutoff){
260       t.resolution <-c(t.end,"no_resolution",NA)
261       }else{
262       t.resolution <-t.passRes[1,c("Date","modality","attribute2")]
263       t.resolution$Date<-as.character(as.Date(t.resolution$Date)+t.cutoff)
264       }
265     }
266   tt.outcome2[i,t.resolutionNames]<-t.resolution
267   }
```

```
268
```

**Listing 26**

Code = "Outcome". Definition of Independent Occurence of Fistula or Stenosis

## .3.4. Independent perforation or abcess occurrence

This code outputs a table with one line per row for each independent perforation event. There may be multiple lines per patient. Perforation, fistula and stenosis events are then combined into one table using the code "outcome_full".

```
1
2  ## 'out' is the boolean vector which describes whether outcome has been met in
       observed procedure
3  ##modality is the modality of the observing procedure
4
5  #create vector of column names of three observation dataframes
6  t.radNames<-colnames(RadAbdo2)
7  t.surgNames<-colnames(Surgery2)
8  t.allNames<-c(t.radNames,t.surgNames)
9
10 tt.out<-t.allNames[grepl("Other$|Perf$",t.allNames)]
11 tt.date<-t.allNames[grepl("Date",t.allNames)]
12 ##'attribute' define outcome attribute (for stenosis this is radiological
       upstream dilatation)
13 attribute<-t.allNames[grepl("StenosisD",t.allNames)]
14 attribute2<-t.allNames[grepl("SurgeryL",t.allNames)]
15
16 ##define colnames of outvector
17 ##LocString is location of outcome eg. '6,7'   (terminal ileum and caecum).
18 t.outNames<-c("id","Date","PerfString","AbcessString","out","modality")
19
20 ##########################
21 ##(merge all modalities)
22 ##########################
23 #RadAbdo2 (perforation or abcess on this exam? c(1,2))
24 grepperfstring_Rad<-grepstring(c(1,2))
25 RadAbdo2$out<-ifelse(!grepl(grepperfstring_Rad,RadAbdo2[,t.radNames%in%tt.out]) |
       is.na(RadAbdo2[,t.radNames%in%tt.out]),0,1)
26 RadAbdo2$modality<-"radiology"
27
28 #Surgery (note need to clean this string, there are a number of '1's recorded.
29 grepperfstring_Surg<-grepstring(c(1,4,5,6,7,8,9,10,11,13))
```

```
grepabcessstring_Surg<-grepstring(c(2,6))
Surgery2$out<-ifelse(grepl(grepperfstring_Surg,Surgery2$SurgeryPerf) | grepl(
    grepabcessstring_Surg,Surgery2$SurgeryOther), 1,0)
Surgery2$modality<-"surgery"

##########################
##select columns of interest only and give them unifying names
##########################
#define function
unify<-function(f.string,f.names,f.table.original){
##f.string -> string on which to search attributes columns to identify which
    table they are in.
##f.names -> string of columnnames of the table to be modified eg "t.surgNames"
##f.table -> name of temporary table to be created eg t.Surgery
##f.table2 -> name of original table eg Surgery
t.test<-!grepl(f.string,attribute)
t.test2<-!grepl(f.string,attribute2)
t.insert<-c("id",f.names[f.names%in%tt.date],f.names[f.names%in%tt.out],"out","
    modality")
if(t.test){
  if(t.test2){
    f.table<-f.table.original[,t.insert]
    f.table$filler<-NA
    f.table$filler2<-NA
    }else{
    f.table<-f.table.original[,t.insert]
    f.table$filler<-NA
    f.table$filler2<-f.table.original[,attribute2]
  }}else{
  if(t.test2){
    t.insert<-c(t.insert,attribute)
    f.table<-f.table.original[,t.insert]
    f.table$filler2<-NA
    }else{
    t.insert<-c(t.insert,attribute,attribute2)
    f.table<-f.table.original[,t.insert]
    }
  }
return(f.table)
}

t.Surgery2<-unify("Surg",t.surgNames,Surgery2)
t.RadAbdo2<-unify("Rad",t.radNames,RadAbdo2)
t.RadAbdo2<-t.RadAbdo2[,c(1:3,3,4:7)]
t.RadAbdo2[,3]<-NA
t.outNames2<-c(t.outNames,"attribute","attribute2")
colnames(t.Surgery2)<-t.outNames2
colnames(t.RadAbdo2)<-t.outNames2
```

```
tt.outcome<-rbind(t.Surgery2,t.RadAbdo2)
tt.outcome<-tt.outcome[!is.na(tt.outcome$Date),]

##remove temp files (those starting with 't.')
rm(list=ls()[grepl("^t\\.",ls())])

#########################
##identify outcomes which qualify as independent outcome events, observed on radiology, colonoscopy or surgery.
#########################

# An old outcome is one that has been identified previously on radiology, colonoscopy or at surgery, prior to time period defined by 'cutoff_passive_independence' and 'cutoff_surgical_independence'.
#   A recent outcome is one identified within time period before the outcome being assessed (as defined by 'cutoff_passive_independence' and 'cutoff_surgical_independence').
# 'cutoff_passive_independence' is used to assess whether most prior outcomes are 'old' or 'recent'.
# If a prior outcome is surgical resection of affected segment,'cutoff_surgical_independence' is used to assess whether it is 'old' or 'recent'.(this cutoff is shorter, acknowledging that surgery usually resolves stenosis/fistulae).
# If no recent outcome exists then the outcome being assessed is considered independent.

tt.outcome2<-data.frame(id=character(),
                 Date=character(),
                 LocString=character(),
    out=character(),
    outcome=character(),
    attribute=character(),
    modality=character(),
                 stringsAsFactors=FALSE)

for (i in 1:length(cd[,1])){
id<-cd$id[i]
t.temp<-tt.outcome[tt.outcome$id==id,]
t.temp<-t.temp[order(t.temp$Date),]

if(length(t.temp[,1])>0){

t.temp$outcome<-NA
t.temp[1,"outcome"]<-t.temp$out[1]
if(length(t.temp[,1])>1){
for (j in 2:length(t.temp[,1])){
##in the 'cutoff_surgical_independence' period before this test, was an outcome observed? (whether or not there has been intervening surgery).
```

```
112 t.compareAll<-subset(t.temp,as.Date(t.temp$Date[j])-as.Date(t.temp$Date)<cutoff_
        surgical_independence & as.Date(t.temp$Date[j])-as.Date(t.temp$Date) > 0 & t.
        temp$out==1)
113
114 ##in the period between 'cutoff_passive_independence' and 'cutoff_surgery_
        independence' before this test, was an outcome which was not "surgery
        resecting involved segment" observed? (If it is a surgical resection in this
        time period it is considered 'old', otherwise it is considered recent).
115 t.comparePassive<-subset(t.temp,as.Date(t.temp$Date[j])-as.Date(t.temp$Date)<
        cutoff_passive_independence & as.Date(t.temp$Date[j])-as.Date(t.temp$Date) >=
         cutoff_surgical_independence & t.temp$out==1 & !t.temp$modality=="surgery")
116 ##code only independent outcomes as '1' in
117 t.temp$outcome[j]<-ifelse(t.temp$out[j]==0 | length(t.compareAll[,1])>0 | length(
        t.comparePassive[,1])>0,0,1)
118 }}}
119 t.temp2<-subset(t.temp,t.temp$outcome==1)
120 tt.outcome2<-rbind(tt.outcome2,t.temp2)
121 }
122 tt.outcome2$outcomeID<-c(1:length(tt.outcome2[,1]))
123
124 ##remove temp files (those starting with 't.')
125 rm(list=ls()[grepl("^t\\.",ls())])
126
127 ##########################
128 #define resolution
129 ##########################
130 ##definition of resolution of outcome:
131 ##subsequent surgery resecting affected segment of bowel within 'surgical_
        resolution_cutoff'; or
132 ##Once "passive_resolution_cutoff' time has passed without recurrent observation
        of stenosis.
133
134 ##add colnames needed for resolution data.
135 t.resolutionNames<-c("DateResolution","resolution","SurgeryLocResolution")
136 for(name in t.resolutionNames){
137 tt.outcome2[,name]<-NA
138 }
139
140 for (i in tt.outcome2$outcomeID){
141   ##reset resolution vector to NA
142   t.resolution<-NA
143   ##id of patient for this outcome event
144   t.id<-tt.outcome2$id[tt.outcome2$outcomeID==i]
145   ##isolate outcome event
146   t.out<-tt.outcome2[tt.outcome2$outcomeID==i,]
147   t.temp<-tt.outcome[tt.outcome$id==t.id,]
148
149
```

```
#determine date of resolution (passive or surgical)
##resMarker: 1 means that this investigation demonstrates that the outcome being observed has not yet resolved: 0 means that it has resolved (over    < passive_resolution_cutoff >    has passed without observation of outcome).
  t.passRes<-t.temp[as.numeric(as.Date(t.temp$Date)-as.Date(t.out$Date)) > 0 & t.temp$out==1 ,]
  t.passRes<-rbind(t.out[,colnames(t.passRes)],t.passRes)
  t.passRes<-t.passRes[order(t.passRes$Date),]
  t.len<-length(t.passRes[,1])
  ##if surgery was affected segment removed?
  t.passRes$resMarker<-NA
  t.passRes$resMarker[1]<-1
  if (length(t.passRes[,1])>1){
    for (j in 2:length(t.passRes[,1])){
      ##if preceding examintion resection of affected segment, use    < surgical_resolution_cutoff1 > otherwise use   < passive_resolution_cutoff >
      if(t.passRes$modality[j-1]=="surgery"){
        t.cutoff<-surgical_resolution_cutoff1
        }else{
        t.cutoff<-passive_resolution_cutoff
        }
      ##if t.cutoff hasn't passed, mark resMarker < 1 >.
      if(as.Date(t.passRes$Date[j])-as.Date(t.passRes$Date[j-1]) <t.cutoff & t.passRes$resMarker[j-1]>0){
        t.passRes$resMarker[j]<-1
        }else{t.passRes$resMarker[j]<-0
        }
      }
    ##mark resMarker2 as < 1 > for the last investigation that observes the unresolved outcome.
    t.passRes$resMarker2<-NA
    for (j in 2:length(t.passRes[,1])){
      if(t.passRes$resMarker[j-1]==1 & t.passRes$resMarker[j]==0){
        t.passRes$resMarker2[j-1]<-1
        }
      }
    ##for the last observation, mark resMarker2 as < 1 > if patient has been observed for < t.cutoff > time and outcome has not been observed again.
    t.cutoff<-NA
    t.end<-cd[cd$id==t.id,"LstSeen"]
    if(is.na(t.end)){t.end<-as.character(Sys.Date())}
    ##if last examintion is resection of affected segment, use    < surgical_resolution_cutoff1 > otherwise use   < passive_resolution_cutoff >
    if(t.passRes$modality[t.len]=="surgery"){
      t.cutoff<-surgical_resolution_cutoff1
      }else{
      t.cutoff<-passive_resolution_cutoff
      }
```

```
190     ##mark resMarker2 as < 1 > for the last investigation if patient has been
        observed for < t.cutoff > time and outcome has not been observed again.
191     if(t.passRes$resMarker[t.len]==1&as.Date(t.end)-as.Date(t.passRes$Date[t.len
        ])>t.cutoff){
192       t.passRes$resMarker2[t.len]<-1
193       }
194     ##select resolution observation ( where resMarker2 = 1 )
195     t.resolution<-subset(t.passRes,!is.na(t.passRes$resMarker2)&t.passRes$
        resMarker2==1)
196     ##assign date of resolution (after appropriate resolution cutoff period has
        passed).
197     if(length(t.resolution[,1])>0){
198       t.resolution<-t.resolution[,c("Date","modality","attribute2")]
199       t.resolution$Date<-ifelse(t.resolution$modality=="surgery",as.character(as.
        Date(t.resolution$Date)+surgical_resolution_cutoff1),as.character(as.Date(t.
        resolution$Date)+passive_resolution_cutoff))
200       }else{
201       t.resolution<-c(t.end,"no_resolution",NA)
202       }
203     }
204   ##above nested code assigns resolution where more than one observation exists
        after the initial observation. For those with only the initial observation,
        code below assigns resolution dependant on whether < resolution cutoff > has
        passed before the end of the observation period.
205   if(!TRUE%in%grepl("[a-z0-9]",t.resolution)){
206     t.end<-cd[cd$id==t.id,"LstSeen"]
207     if(is.na(t.end)){t.end<-as.character(Sys.Date())}
208     if(t.passRes$modality[1]=="surgery"){
209       t.cutoff<-surgical_resolution_cutoff1
210       }else{
211       t.cutoff<-passive_resolution_cutoff
212       }
213     if(as.Date(t.end)-as.Date(t.passRes$Date)<t.cutoff){
214       t.resolution <-c(t.end,"no_resolution",NA)
215       }else{
216       t.resolution <-t.passRes[1,c("Date","modality","attribute2")]
217       t.resolution$Date<-as.character(as.Date(t.resolution$Date)+t.cutoff)
218       }
219     }
220   tt.outcome2[i,t.resolutionNames]<-t.resolution
221   }
```

**Listing 27**

Code = “outcome_abcess_perf”. Definition of Independent Occurence of Perforation

## .3.5. Merging of fistula, stenosis or perforation events

This code combines seperate tables made for fistulae, perforation events and stenosis events, and combines them into one outcome table. Where different events (fistula and stensis for eg.) occur at the same time, they are combined into a "combination" event.

```
###merge into one outcome dataframe
outcome.fistula$outcome<-"fistula"
outcome.perforation$outcome<-"perforation"
outcome.stenosis$outcome<-"stenosis"
cols<-c("id","Date","DateResolution","outcome","resolution","SurgeryLocResolution")
outcome<-rbind(outcome.fistula[,cols],outcome.perforation[,cols],outcome.stenosis[,cols])
outcome<-outcome[order(outcome$id,outcome$Date,outcome$DateResolution),]
table(outcome$id)

##add a row for each patient to outcome_merged (for analysis to censoring at end of study period)
cols<-colnames(outcome)
colsCd<-c("id","LstSeen","DateDiag1")
temp<-cd[,colsCd]
temp$DateDiag1<-NA
colnames(temp)<-cols[c(1:3)]
temp[,cols[4:length(cols)]]<-NA
temp$outcome<-"no_outcome:censor"
outcome<-rbind(temp,outcome)
outcome$start<-NA
test<-!is.na(outcome$Date)
outcome<-subset(outcome,test)

##if outcomes overlap, merge them.
##this includes censored segements at end of dataset
##cutoff is minumum gap between two outcomes in days
cutoff<-0
outcome_merged<-outcome[0,]
t.outcome<-outcome[!duplicated(outcome$id),]
for(i in 1:length(t.outcome$id)){
id<-t.outcome$id[i]
lstSeen<-cd$LstSeen[cd$id==id]
temp<-outcome[outcome$id==id & as.Date(outcome$Date)<=as.Date(lstSeen),]
temp<-temp[order(temp$Date),]
  if(length(temp$id)>1){
```

```
    for (j in 2:length(temp$id)){
      latest<-as.character(max(as.Date(temp$DateResolution[c((j-1):j)]),na.rm=
    TRUE))
      temp2<-temp[(j-1):j,]
      test<-temp2$DateResolution==latest
      test<-ifelse(is.na(test),FALSE,test)
      latestResolution<-temp2[test,"resolution"]
      latestSurgeryLocResolution<-temp2[test,"SurgeryLocResolution"]

      if(length(latestResolution)>1){
        latestResolution<-latestResolution[1]
        latestSurgeryLocResolution<-latestSurgeryLocResolution[1]}
        if(!is.na(as.Date(temp$DateResolution[j-1]))){
          if(as.Date(temp$Date[j])<=as.Date(temp$DateResolution[j-1])+cutoff){
            temp[j,c("Date","DateResolution","outcome","resolution")]<-c(as.
    character(as.Date(temp$Date[j-1])),latest,paste(temp$outcome[j-1],temp$
    outcome[j]),latestResolution)
            temp[j-1,]<-NA
            }
          }
        }
      }
  temp<-temp[!is.na(temp$id),]
  outcome_merged<-rbind(outcome_merged,temp)
  print(i)
  }

outcome_merged$outcome2<-gsub("[[:space:]]no_outcome:censor","",outcome_merged$
    outcome)

##define periods leading up to analysis for outcome
##start is start of analysis period: either diagnosis date + "observation_cut_
    diag" or date of resolution of prior outcome -  "observation_cut_outcome"
outcome_full<-outcome_merged[0,]
t.outcome<-outcome_merged[!duplicated(outcome_merged$id),]
for(i in 1:length(t.outcome$id)){
id<-t.outcome$id[i]
temp<-outcome_merged[outcome_merged$id==id,]
  for(j in 1:length(temp[,1])){
  start<-temp$DateResolution[temp$DateResolution<=temp$Date[j]]
    if(TRUE%in% !is.na(start)){
    start<-as.character(max(as.Date(start),na.rm=TRUE)-observation_cut_outcome)
        }else{
    start<-as.character(as.Date(Diag$DateDiag[Diag$id==id])+observation_cut_diag)
    if(TRUE%in% !is.na(start)){
    start<-as.character(min(as.Date(start),na.rm=TRUE))
    }else{
    start<-NA
```

```
    }
    }
  temp$start[j]<-start
  }
outcome_full<-rbind(outcome_full,temp)
}

##restrict analysis to where observation period is greater than < observation_cut
    _diag >
outcome_full$ShortObs<-as.Date(outcome_full$Date)-as.Date(outcome_full$start)<
    observation_cut_diag

##exclude outcomes which occur within 6 months of diagnosis.
outcome_full$DiagWithinSix<-NA
for(i in 1:length(outcome_full[,1])){
id<-outcome_full$id[i]
test<-cd$id==id
t.dateDiag<-cd$DateDiag1[test]
test2<-!(as.Date(outcome_full$Date[i])-as.Date(t.dateDiag)>observation_cut_diag)
outcome_full$DiagWithinSix[i]<-test2
}

##exclude outcomes which occur within 12 months of diagnosis.
outcome_full$DiagWithin12<-NA
for(i in 1:length(outcome_full[,1])){
id<-outcome_full$id[i]
test<-cd$id==id
t.dateDiag<-cd$DateDiag1[test]
test2<-!(as.Date(outcome_full$Date[i])-as.Date(t.dateDiag)>365)
outcome_full$DiagWithin12[i]<-test2
}
```

**Listing 28**

Definition of Independent Occurence of Fistula, Stenosis or Peroforation: Merging observations of same complications

## .4. Perianal fistula formation

Perianal fistulae were defined using data taken from dated perianal surgical procedures, dated perianal imaging procedures and dated clinical examinations of the perineum. The following code defines how these data were transformed into a

table which tabulates temporal progression from date of diagnosis to perianal fistula formation, and when this fistula was deemed to have clinically resolved. The resulting table is labelled *outcome_full* and has one or many lines per patient.

```
## 'out' is the boolean vector which describes whether outcome has been met in observed procedure
##modality is the modality of the observing procedure

obs<-"Fist"

#create vector of column names of three observation dataframes
t.clinNames<-colnames(PAclinical)
t.surgNames<-colnames(PASurgery)
t.radNames<-colnames(RadPA)
t.allNames<-c(t.clinNames,t.radNames,t.surgNames)

tt.out<-t.allNames[grepl(obs,t.allNames)]
tt.date<-t.allNames[grepl("Date",t.allNames)]
##'attribute' define outcome attribute (for stenosis this is radiological upstream dilatation)
#attribute<-t.allNames[grepl("StenosisD",t.allNames)]
#attribute2<-t.allNames[grepl("SurgeryL",t.allNames)]

##define colnames of outvector
##LocString is location of outcome eg. '6,7'   (terminal ileum and caecum).
t.outNames<-c("id","Date","RadModality","Fistula","out","modality")

##clean outcome field of interest (no text)
#note will need to review fields in this coding at a later date.
##change 1 to 0 if it exists on it's own in stenosis strings (likely coding error will need to be reviewed at a later date).
PASurgery[,t.surgNames%in%tt.out]<-ifelse(grepl("[a-zA-Z]",PASurgery[,t.surgNames%in%tt.out]),NA,PASurgery[,t.surgNames%in%tt.out])

##########################
##(merge all modalities)
##########################
#RadPA
RadPA$out<-ifelse(!(RadPA[,t.radNames%in%tt.out]==0 | is.na(RadPA[,t.radNames%in%tt.out])),1,0)
RadPA$modality<-"radiology"
t.RadPA<-RadPA[,c("id","RadPADate","RadPA","RadPAFistula","out","modality")]
#Clinical Exam
PAclinical$out<-ifelse(!(PAclinical[,t.clinNames%in%tt.out]==0 | is.na(PAclinical[,t.clinNames%in%tt.out])),1,0)
PAclinical$modality<-"clinical exam"
```

```
PAclinical$fill<-NA
t.PAclinical<-PAclinical[,c("id","PAClinicalDate","fill","PAClinicalFistula","out","modality")]
#Surgery
PASurgery$out<-ifelse(!(PASurgery[,t.surgNames%in%tt.out]==0 | is.na(PASurgery[,t.surgNames%in%tt.out])),1,0)
PASurgery$modality<-"surgery"
PASurgery$fill<-NA
t.PASurgery<-PASurgery[,c("id","PASurgeryDate","fill","PASurgeryFistula","out","modality")]

colnames(t.PAclinical)<-t.outNames
colnames(t.PASurgery)<-t.outNames
colnames(t.RadPA)<-t.outNames
tt.outcome<-rbind(t.PAclinical,t.PASurgery,t.RadPA)
tt.outcome<-tt.outcome[!is.na(tt.outcome$Date),]

##remove temp files (those starting with 't.')
rm(list=ls()[grepl("^t\\.",ls())])

#########################
##identify outcomes which qualify as independent outcome events, observed on radiology, colonoscopy or surgery.
#########################

# An old outcome is one that has been identified previously on radiology, colonoscopy or at surgery, prior to time period defined by 'cutoff_passive_independence' and 'cutoff_surgical_independence'.
#   A recent outcome is one identified within time period before the outcome being assessed (as defined by 'cutoff_passive_independence' and 'cutoff_surgical_independence').
# 'cutoff_passive_independence' is used to assess whether most prior outcomes are 'old' or 'recent'.
# If a prior outcome is surgical resection of affected segment,'cutoff_surgical_independence' is used to assess whether it is 'old' or 'recent'.(this cutoff is shorter, acknowledging that surgery usually resolves stenosis/fistulae).
# If no recent outcome exists then the outcome being assessed is considered independent.


tt.outcome2<-data.frame(id=character(),
                 Date=character(),
                 LocString=character(),
    out=character(),
    outcome=character(),
    attribute=character(),
    modality=character(),
                 stringsAsFactors=FALSE)
```

```
73
74 for (i in 1:length(cd[,1])){
75 id<-cd$id[i]
76 t.temp<-tt.outcome[tt.outcome$id==id,]
77 t.temp<-t.temp[order(t.temp$Date),]
78
79 if(length(t.temp[,1])>0){
80
81 t.temp$outcome<-NA
82 t.temp[1,"outcome"]<-t.temp$out[1]
83 if(length(t.temp[,1])>1){
84 for (j in 2:length(t.temp[,1])){
85 ##in the 'cutoff_surgical_independence' period before this test, was an outcome
       observed? (whether or not there has been intervening surgery).
86 t.comparePassive<-subset(t.temp,as.Date(t.temp$Date[j])-as.Date(t.temp$Date)<
       cutoff_passive_independence & as.Date(t.temp$Date[j])-as.Date(t.temp$Date) >
       0 & t.temp$out==1)
87 ##code only independent outcomes as '1' in
88 t.temp$outcome[j]<-ifelse(t.temp$out[j]==0 | length(t.comparePassive[,1])>0,0,1)
89 }}}
90 t.temp2<-subset(t.temp,t.temp$outcome==1)
91 t.temp2<-subset(t.temp2,!duplicated(paste(t.temp2$id,t.temp2$Date)))
92 tt.outcome2<-rbind(tt.outcome2,t.temp2)
93 }
94 tt.outcome2$outcomeID<-c(1:length(tt.outcome2[,1]))
95
96 ##remove temp files (those starting with 't.')
97 rm(list=ls()[grepl("^t\\.",ls())])
98
99 ##########################
100 #define resolution
101 ##########################
102 ##definition of resolution of outcome:
103 ##subsequent surgery resecting affected segment of bowel within 'surgical_
       resolution_cutoff'; or
104 ##Once "passive_resolution_cutoff' time has passed without recurrent observation
       of stenosis.
105
106 ##add colnames needed for resolution data.
107 t.resolutionNames<-c("DateResolution","resolution")
108 for(name in t.resolutionNames){
109 tt.outcome2[,name]<-NA
110 }
111
112 for (i in tt.outcome2$outcomeID){
113   ##reset resolution vector to NA
114   t.resolution<-NA
115   ##id of patient for this outcome event
```

```
116   t.id<-tt.outcome2$id[tt.outcome2$outcomeID==i]
117   ##isolate outcome event
118   t.out<-tt.outcome2[tt.outcome2$outcomeID==i,]
119   t.temp<-tt.outcome[tt.outcome$id==t.id,]
120
121 #determine date of resolution (passive or surgical)
122 ##resMarker: 1 means that this investigation demonstrates that the outcome being
      observed has not yet resolved: 0 means that it has resolved (over    <
      passive_resolution_cutoff >    has passed without observation of outcome).
123   t.passRes<-t.temp[as.numeric(as.Date(t.temp$Date)-as.Date(t.out$Date)) > 0 & t.
      temp$out==1 ,]
124   t.passRes<-rbind(t.out[,colnames(t.passRes)],t.passRes)
125   t.passRes<-t.passRes[order(t.passRes$Date),]
126   t.len<-length(t.passRes[,1])
127   t.passRes$resMarker<-NA
128   t.passRes$resMarker[1]<-1
129   if (length(t.passRes[,1])>1){
130     for (j in 2:length(t.passRes[,1])){
131       t.cutoff<-passive_resolution_cutoff
132       ##if t.cutoff hasn't passed, mark resMarker < 1 >.
133       if(as.Date(t.passRes$Date[j])-as.Date(t.passRes$Date[j-1]) <t.cutoff & t.
      passRes$resMarker[j-1]>0){
134         t.passRes$resMarker[j]<-1
135         }else{t.passRes$resMarker[j]<-0
136         }
137       }
138     ##mark resMarker2 as < 1 > for the last investigation that observes the
      unresolved outcome.
139     t.passRes$resMarker2<-NA
140     for (j in 2:length(t.passRes[,1])){
141       if(t.passRes$resMarker[j-1]==1 & t.passRes$resMarker[j]==0){
142         t.passRes$resMarker2[j-1]<-1
143         }
144       }
145     ##for the last observation, mark resMarker2 as < 1 > if patient has been
      observed for < t.cutoff > time and outcome has not been observed again.
146     t.cutoff<-NA
147     t.end<-cd[cd$id==t.id,"LstSeen"]
148     if(is.na(t.end)){t.end<-as.character(Sys.Date())}
149     t.cutoff<-passive_resolution_cutoff
150     if(t.passRes$resMarker[t.len]==1&as.Date(t.end)-as.Date(t.passRes$Date[t.len
      ])>t.cutoff){
151       t.passRes$resMarker2[t.len]<-1
152       }
153     ##select resolution observation ( where resMarker2 = 1 )
154     t.resolution<-subset(t.passRes,!is.na(t.passRes$resMarker2)&t.passRes$
      resMarker2==1)
```

```
155   ##assign date of resolution (after appropriate resolution cutoff period has
        passed).
156     if(length(t.resolution[,1])>0){
157       t.resolution<-t.resolution[,c("Date","modality")]
158       t.resolution$Date<-as.character(as.Date(t.resolution$Date)+passive_
      resolution_cutoff)
159       }else{
160       t.resolution<-c(t.end,"no_resolution")
161       }
162     }
163 ##above nested code assigns resolution where more than one observation exists
      after the initial obse  rvation. For those with only the initial observation,
       code below assigns resolution dependant on whether < resolution cutoff > has
       passed before the end of the observation period.
164   if(!TRUE%in%grepl("[a-z0-9]",t.resolution)){
165     t.end<-cd[cd$id==t.id,"LstSeen"]
166     if(is.na(t.end)){t.end<-as.character(Sys.Date())}
167     t.cutoff<-passive_resolution_cutoff
168     if(as.Date(t.end)-as.Date(t.passRes$Date)<t.cutoff){
169       t.resolution <-c(t.end,"no_resolution")
170       }else{
171       t.resolution <-t.passRes[1,c("Date","modality")]
172       t.resolution$Date<-as.character(as.Date(t.resolution$Date)+t.cutoff)
173       }
174     }
175   tt.outcome2[i,t.resolutionNames]<-t.resolution
176   }
177
178 outcome<-tt.outcome2
179
180 ##add a row for each patient to outcome_merged (for analysis to censoring at end
      of study period)
181 cols<-colnames(outcome)
182 colsCd<-c("id","LstSeen","DateDiag1")
183 temp<-cd[,colsCd]
184 temp$DateDiag1<-NA
185 colnames(temp)<-cols[c(1:3)]
186 temp[,cols[4:length(cols)]]<-NA
187 temp$outcome<-"no_outcome:censor"
188 outcome<-rbind(temp,outcome)
189 outcome$start<-NA
190 test<-!is.na(outcome$Date)
191 outcome<-subset(outcome,test)
192
193 ##define periods leading up to analysis for outcome
194 ##start is start of analysis period: either diagnosis date + "observation_cut_
      diag" or date of resolution of prior outcome -  "observation_cut_outcome"
195 outcome_full<-outcome[0,]
```

```
t.outcome<-outcome[!duplicated(outcome$id),]
for(i in 1:length(t.outcome$id)){
id<-t.outcome$id[i]
temp<-outcome[outcome$id==id,]
  for(j in 1:length(temp[,1])){
  start<-temp$DateResolution[temp$DateResolution<=temp$Date[j]]
    if(TRUE%in% !is.na(start)){
    start<-as.character(max(as.Date(start),na.rm=TRUE)-observation_cut_outcome)
    }else{
    start<-as.character(as.Date(Diag$DateDiag[Diag$id==id])+observation_cut_diag)
    if(TRUE%in% !is.na(start)){
    start<-as.character(min(as.Date(start),na.rm=TRUE))
    }else{
    start<-NA
    }
    }
  temp$start[j]<-start
  }
outcome_full<-rbind(outcome_full,temp)
}

#outcome_full<-subset(outcome_full,!(outcome_full$DiagWithinSix | outcome_full$
    ShortObs))
```

**Listing 29**

Definition of Independent Occurence of Perianal Fistulae

## .5. Smoking status

This function defines whether or not a patient was smoking at a defined timepoint.

```
fun_smoking<-function(dataframe,datefield){
##Smoking status at datefield
#0=never, 1=ex-smoker, 2=current
t.age.stop<-ifelse(dataframe[,"SmokeStopAge"]=="CURRENT",100,dataframe[,"
    SmokeStopAge"])
smoke<-ifelse(dataframe[,"SmokeEver"]==0,0,
ifelse((as.Date(dataframe[,"DOB"])+365*as.numeric(dataframe[,"SmokeStartAge"]))>
    as.Date(dataframe[,datefield]),0,
ifelse((as.Date(dataframe[,"DOB"])+365*as.numeric(t.age.stop))>as.Date(dataframe
    [,datefield]),2,1)))
return(smoke)
}
```

**Listing 30**

Definition of smoking status